ACTIVATED SLUDGE PROCESS:
EFFECTS OF FEED CONCENTRATION ON EFFLUENT COD

by

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ABSTRACT

The concentration of substrates in the feed to an activated sludge process was found to exert a significant effect upon its effluent COD. A mathematical model was proposed to explain this effect and was successful in correlating the data of this study. The model was based on the hypothesis that COD measures both substrate and product concentrations. It was found that an optimum sludge age exists for achieving minimum effluent COD. At sludge ages longer than the optimum, effluent COD increased due to product formation; at shorter sludge ages the effluent COD increased due to an increased concentration of degradable substrate.

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NOMENCLATURE

BOD_5	biochemical oxygen demand (5 day test) (mg/ℓ)
b	specific decay rate ($days^{-1}$)
C_1	constant defined in Equation (3.32) ($\ell mg^{-1}\ d^{-1}$)
COD	chemical oxygen demand (mg/ℓ)
F	feed rate (ℓ/h)
G	flow rate (ℓ/h)
f	parameter defined in Equation (3.36)
g	parameter defined in Equation (3.37)
g.f.	glass fibre filter paper
h	parameter defined in Equation (3.38)
K_s	saturation constant (mg/ℓ)
K^1	parameter defined in Equation (5.13)
K^{11}	parameter defined in Equation (5.13)
k_d	death constant ($hours^{-1}$, $days^{-1}$)
manucol	sodium alginate
milli	millipore filter paper
mixed	50% by COD manucol and 50% by COD sugar
MLSS	mixed liquor suspended solids
MLVSS	mixed liquor volatile suspended solids
P	product concentration (mg/ℓ)
Q	flow rate (ℓ/h)
R_H	hydraulic retention time (hours, days)
R_S	sludge age (hours, days)
r	rate term (mg/ℓ-hour)
S	concentration of growth limiting substrate (mg/ℓ)
So	feed concentration (mg/ℓ)
SVI	sludge volume index

t	time (hours, days)
V	volume (ℓ)
W	sludge wastage rate (ℓ/h)
X	concentration of microorganisms (mg/ℓ)
X_e	effluent cell concentration (mg/ℓ)
X_o	initial cell concentration (mg/ℓ)
X_r	cell concentration in recycle stream (mg/ℓ)
Y	yield coefficient (mg-cells/mg-product)
α	constant (mg-product mg-cells^{-1})
β	constant (mg-product mg-cells^{-1} d^{-1})
μ	specific growth rate (hours^{-1}, days^{-1})
μ_m	maximum specific growth rate (hours^{-1}, days^{-1})

Subscripts and Superscripts:

e	endogenous metabolism
g	growth
min	minimum
opt	optimum
p	product
s	substrate
x	cells

CHAPTER 1

INTRODUCTION

An efficient method for reducing the organic content of liquid effluent is by aerobic biological treatment. A variety of organic compounds are found in liquid wastes, ranging from readily degradable carbohydrates to relatively undegradable materials. It is usually difficult to determine the concentration of these compounds individually, and chemical or biological oxygen demands (COD, BOD) are used as measures of waste strength.

Microorganisms need oxygen and nutrients, as well as a carbon source, to grow. In waste water treatment processes oxygen and nutrients are provided in sufficient quantities to ensure that they do not limit growth. The growth of micro-organisms is then dependent upon the carbon concentration. For a single substrate, the growth rate of microorganisms can be described by the Monod [1] function, namely

$$\mu = \frac{\mu_m S}{K_s + S} \qquad (1.1)$$

where μ = specific growth rate of microorganisms

μ_m = maximum specific growth rate

S = concentration of growth limiting substrate (i.e. carbon source)

K_s = saturation constant, numerically equal to the substrate concentration at $\mu = \mu_m/2$.

When an effluent contains more than one carbon source, it is assumed that COD is a measure of the growth limiting substrate, i.e. S = COD in Equation (1.1).

It is unlikely that this is generally true. Depending on the conditions of growth, different substrates may be limiting at different times. For instance, it has been demonstrated that microorganisms can preferentially assimilate one substrate at a time, temporarily preventing the metabolism of the others [2]. The assumption, therefore, that the Monod function expresses the relationship between growth rate of

microorganisms and COD of an effluent, may not apply.

In order to investigate this assumption a waste water was simulated in this study by a two-substrate medium; one substrate represented the readily degradable fraction and the other the less readily degradable fraction of a waste water. Laboratory-scale activated sludge units were operated on each of the two substrates individually and on the two substrates simultaneously, to see whether the growth rate versus COD relationships followed Monod kinetics, and whether the relationships for the individual substrates could be used to predict the mixed substrate behaviour.

COD-growth rate relationships were obtained for single and mixed substrates which could not be explained by Monod theory. This was attributed to the formation of products during substrate metabolism. A mathematical model for product formation was proposed and incorporated in activated sludge theory, and used to interpret the results of experimental investigations.

It is proposed that the model be used to describe the activated sludge process where COD measures waste strength.

CHAPTER 2

LITERATURE SURVEY

In the first part of the chapter the bacterial assimilation of multiple and single substrates is reviewed. Product formation, which was observed in this investigation, is discussed in the second part of the chapter.

2.1 DUAL (AND MULTIPLE) SUBSTRATE EXPERIMENTS

There are many papers in the literature which describe batch experiments on two or more substrates. Reports that describe the occurrence of sequential substrate utilization (diauxic growth) are of interest to this investigation, as they suggest that the use of COD as a measure of the growth limiting substrate of a waste water is not valid.

Monod [1] found that when a single microorganism was present with several carbon sources, growth took place on the best carbon source, then on the second best, and so on. Davis [3] showed that a carbohydrate need be present in only small concentrations to block the growth response to another. Hamilton and Dawes [4,5] found that the substrate metabolism which was suppressed was dependent on the organism used: for example, in some experiments glucose was used before citrate [6,7]; in others citrate before glucose [4,5].

Gaudy [2] demonstrated that diauxic growth can occur with a heterogeneous population. He found that glucose was used before sorbitol was attacked, irrespective of the substrate to which the population had been acclimatised. A later study [8] showed that cell age was a variable which influenced diauxie: cells grown at long sludge ages removed glucose and sorbitol simultaneously. Carter and Malaney [9] conducted batch experiments on chemicals found in a typical waste stream, e.g. alkanes, keytones, amino acids and aromatic hydrocarbons. They found that, for most experiments in which the sludge was exposed to two substrates, there was preferential oxidation of

one of the two compounds.

Diauxic growth occurs in continuous as well as batch cultures. Chian and Mateles found that glucose interfered with the uptake of fructose, lactose or butyric acid at short sludge ages, while at long sludge ages the sugars were consumed simultaneously [10, 11, 12]. In all cases substantial excretion of acetate, amounting to 10 to 20% of the carbon source consumed, was observed at moderate to short sludge ages. Acetate excretion was also recorded [11] when a heterogeneous batch culture was grown on glucose alone. The acetate was attacked only after the glucose had been completely consumed.

Diauxic growth does not always occur when there is more than one substrate available to the microorganisms. Eckenfelder and Tischler [13] found that glucose, aniline and phenol were all removed simultaneously, and the rate of removal of each compound was independent of the presence of the other. Ghosh [14] found no evidence of diauxic growth when a heterogeneous continuous culture was grown on glucose and galactose. Instead the galactose consumption was enhanced by the presence of glucose. Both the glucose and galactose concentrations were found to be hyperbolic functions of the growth rate.

Jones [15] considered mathematically a mixture of 10 substrates and assumed that each component was independently metabolised by a specific population of organisms. He assigned to each substrate an influent concentration and its own kinetic constants in the ranges $Y = 0,11 - 0,49$, $\mu_m = 0,17 - 0,9\ h^{-1}$ and $K_s = 3 - 19$ mg/ℓ. The total substrate concentration, which is analogous to a parameter like COD, was calculated for a range of sludge ages. A Lineweaver and Burke [16] plot gave an excellent fit to the data, and the results indicated that for the system the kinetic constants were $\mu_m = 0,311\ h^{-1}$ and $K_s = 85$ mg/ℓ. The K_s value for the system was more than 4 times higher than the highest K_s value for any individual substrate. This is consistent with the high K_s values that are often reported for waste waters, where COD is used as a measure of the organic strength.

2.2 SINGLE SUBSTRATE EXPERIMENTS

Recently several papers have been published describing experiments in which glucose was used as sole substrate. A feature of these papers is that they all report a residual COD in the effluent, even at long sludge ages. No adequate explanation has been presented to explain this residue, but it is probably due to intermediate product formation, which is discussed in Section 2.3.

Chiu et al. [17, 18] evaluated several kinetic models, and found that their steady state results could be adequately fitted with the models of Monod [1], Moser [19] and Contois [20], each with an endogenous term added. They conducted batch experiments on cells obtained at each steady state, and found that μ_m and K_s values were high at short sludge ages and low at long sludge ages [18]. They evaluated the validity of their "steady state" data in long term continuous culture experiments, and found large variations in cell concentrations at each steady state, which they attributed to the heterogeneity of the population [17]. At sludge ages near washout, multiple steady states were observed. (For example, at R_s of 1,75 hours, 3 steady state MLSS were recorded, at 260, 460 and 550 mg/ℓ). The COD was also considerably different for each apparent steady state. Generally, the MLSS and COD results had higher variations at shorter sludge ages than at longer sludge ages.

Gaudy, Ramanathan and Rao [21] confirmed the findings of Chiu et al. [17, 18]. They found that at a constant sludge age the variation in effluent COD was not excessive but there was considerable variation in the biological solids concentration. They suggest that this variation is to be expected when using heterogeneous populations. They found that the variation in kinetic constants was due to selectivity for species with the highest growth rate at each sludge age, and this resulted in a change in predominant species. The experiments were repeated [22] at a higher feed strength and these confirmed the previous findings.

Slime growth on the surface of reactors can also produce erratic fluctuations in substrate and cell concentrations. Ghosh [14] found that all his steady states were characterised by substrate and cell concentrations oscillating about a mean. He found it difficult to get a steady state at a sludge age of less than 3 hours. His results indicate that at long sludge ages the culture was dominated by species with low μ_m and K_s values. At short sludge ages, the dominant species had higher μ_m and K_s values.

It has been shown in recent studies that effluent COD is a function of feed strength. Gaudy and Srinivasaraghaven [23] investigated the activated sludge treatment of glucose at three feed concentrations. The unit had a sludge recycle stream and the recycle cell concentration was kept constant. Reasonably steady COD and cell concentrations were measured. It was found that the average effluent COD increased as feed COD increased.

Grady, Harlow and Riesing [24] used pure cultures in chemostats with glucose as the sole carbon and energy source. They found that at a constant sludge age, the glucose concentration was independent of the feed strength (S_o), but the COD changed as S_o changed. The COD was always greater than the equivalent glucose concentration. Both the COD and glucose concentrations were functions of the sludge. When heterogeneous cultures were used, neither the COD nor the glucose concentrations were constant for a given sludge age. The species which predominated appeared to be influenced by both the sludge age and S_o. Grady and Williams [25] confirmed that feed strength exerts a significant effect upon effluent COD.

A summary of kinetic constants reported for glucose is given in Table 2.1. It is evident that application of conventional activated sludge theory yields kinetic constants which can differ by an order of magnitude.

TABLE 2.1

KINETIC CONSTANTS FOR A HETEROGENEOUS POPULATION GROWN ON GLUCOSE

SOURCE	μ_m (h^{-1})	K_s (mg/ℓ)	Y	k_d,b (h^{-1})	MODE
Peil and Gaudy [26]	0,49	29			batch
	0,38	11			batch
Chiu et al. [17,18]	0,69	27	0,58	k_d=0,019	continuous
	0,23-0,95	0-246	0,48-0,71	k_d=0,006 to 0,024	batch
Gaudy and Srinivasaraghaven [23]	0,49	115	0,39		batch
	0,55	285	0,50		batch
	0,58	105	0,56		batch
Gaudy et al. [21 22]	0,55	90	0,65		continuous
	0,52	57	0,57		continuous
	0,47	67	0,70		continuous + recycle
Ghosh [14]	0,728	1,7	0,84	b=0,116	continuous
	1,411	60	0,84		

2.3 PRODUCT FORMATION

As shown in Table 2.2, several workers have measured a residual COD which is probably due to the formation of products. Several of the workers [17, 23, 24] found that the COD was always higher than the equivalent glucose concentration, indicating excretion of metabolic products into the medium. Chiu et al. [17] believed these metabolic substances to be non-biodegradable. Similarly, Gaudy and Srinivasaraghaven [23] reported that, although the exact nature of the residual COD is not known, only 10 - 15% was due to carbohydrates. They believed that the material most likely consisted of various cell components which had leaked from the viable cells, or were components of damaged or dead cells in the sludge, and that the residual COD consisted of material which was more slowly metabolised than the original carbon source in the waste medium.

Eckenfelder [27], quoting the results of Chudoba [28, 29], indicated that the biological oxidation of degradable organic compounds yielded refractory organics as a by-product. This resulted in a residual COD, which was made up of non-degradable materials in the feed and residual compounds resulting from cell lysis and auto-oxidation. It was found [28] that, while the effluent BOD_5 remained relatively constant with increasing feed strength, the effluent COD increased due to increased non-biodegradable products of oxidation. Furthermore, the residual COD increased as the sludge age increased as a result of the release of refractory organics into the solution [29]. At a sludge age of 10 days the residual soluble COD was 20 mg/ℓ. For a sludge age of 50 days the residual COD was 40 mg/ℓ. The soluble BOD was constant over this sludge age range. Eckenfelder [30] reported that the residual BOD or COD remained in the effluent even after long periods of aeration. He indicated that the residue was due to resolubilization of cellular material during cell auto-oxidation. Chian and Mateles [10, 11] reported that the metabolites formed during growth on monosaccharides contain acetic acid. Chian and De Walle [31] observed the excretion of intermediates in a heterogeneous batch culture grown on a complex waste water.

TABLE 2.2

LOWEST EFFLUENT COD VALUES RECORDED DURING CONTINUOUS HETEROGENEOUS CULTURE ON GLUCOSE

SOURCE	FEED CONC. mg/ℓ	RESIDUAL COD mg/ℓ	MODE
Gaudy et al. [21,22]	1 000	45	continuous
	3 000	87	continuous
Chiu et al. [17,18]	1 000	23	"
Grady et al. [24]	500	21	"
	1 000	38	"
	1 500	70	"
	2 000	84	"
Grady and Williams [25]	500	13	"
	2 000	46	"
Gaudy and Srinivasaraghaven [23]	500	25	continuous plus recycle
	1 000	33	
	2 000	38	

They found that amino acids were another major product excreted into the medium.

Gaudy and Gaudy [32] point out that the release of metabolic intermediates during biological treatment could convert an industrial waste with only a few carbon sources to a much more heterogeneous mixture of compounds which could seriously affect the overall purification efficiency. Their findings indicate a need for caution in studies where purification is determined by analysing for specific substrates. Grady et al. [24] indicate that the use of COD as a measure of the purification efficiency can lead to difficulties because, if COD is used as a measure of the growth limiting nutrient, the results do not follow the limiting nutrient - growth rate concept of Monod [1].

Luedeking and Piret [33] studied the fermentation of glucose to lactic acid, and they advanced the following model for product formation:

$$\frac{dP}{dt} = \alpha\left(\frac{dX}{dt}\right)_g + \beta X \qquad (2.1)$$

where P and X are product and cell concentrations respectively, and α and β are constants. The term involving α is the growth associated rate of product formation, i.e. product that is formed as a by-product of cell growth. The term involving β is the nongrowth associated rate of product formation, i.e. product that is formed as a by-product of cell auto-oxidation.

This concept of product formation was incorporated into the kinetic description of a biological waste treatment process by Eckhoff and Jenkins [34]. They assumed that the rate of product formation could be described by a growth associated model, i.e. the above equation with $\beta = 0$.

2.4 SUMMARY

It is difficult to determine precise values for the kinetic constants of a natural culture because the predominant microbial species changes. The system is even more complicated when there is more than one substrate present, as the growth

of microorganisms on a second substrate can be affected to the point at which no growth occurs. Even when a single substrate is metabolised, especially if it is a monosaccharide, intermediate products are formed, and these often have a growth rate significantly less than the original compound.

2.5 SUBSTRATES USED IN THIS INVESTIGATION

Commercial sugar and manucol were used in the experiments reported here, and this section provides some background information on these substrates.

2.5.1 Sugar

Peil and Gaudy [26] determined the kinetic constants for heterogeneous batch cultures on a variety of substrates, including sugar. When they repeated their experiments after an interval of several weeks, they found significant differences in the results (for sugar, $\mu_m = 0,55\ h^{-1}$, $K_s = 17\ mg/\ell$; on repeating the experiment they obtained $\mu_m = 0,28\ h^{-1}$, $K_s = 6\ mg/\ell$). They concluded that in a heterogeneous population the predominant species varied, and a usable range rather than a precise value for the constants may be determined.

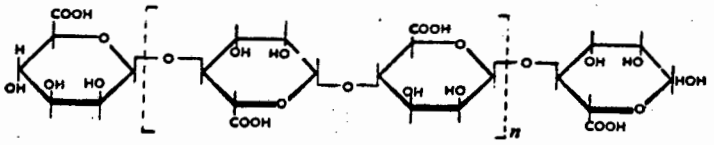
2.5.2 Manucol (Sodium Alginate)

The alginates are naturally occuring substances which are found in brown seaweeds [35]. They are widely used in food products because of their strong stabilizing properties.

The generally accepted structure of alginic acid is that it is a polymer of anhydro-mannuronic and -guluronic acid, as shown in Figure 2.1.

FIGURE 2.1

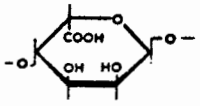
STRUCTURE OF THE TWO FORMS OF ALGINIC ACID



ALGINIC ACID (with D-Mannuronic acid units)

or with some

L-Guluronic acid units:-



It is not known whether it is a heteropolymer comprising the two uronic acids or whether it consists of two different polymers, one made up entirely of mannuronic acid and the other solely of guluronic acid [36].

Güde and Reichardt [37] have indicated that as yet the kinetics of the microbial decomposition of compounds of biological origin like polysaccharides represent a rather unknown subject in water ecology. Schulzen [38] noted that Alginomonas and Alginobacter are capable of degrading acid. These are gram-negative motile rods. While Alginobacter can decompose glucose, Alginomonas cannot utilize carbohydrates [39]. Lynn et al. [40] showed that the principal mechanism involved in bacterial degradation of an alginate was via an eliminative cleavage of the glycosidic linkages. All species of Arthrobacter studied utilized alginate as a source of carbon and energy. Of the 43 Phycomycetes, Ascomycetes and Actinomycetes examined, none were able to utilize alginic acid as a source of carbon and energy.

CHAPTER 3

MATHEMICAL MODEL OF THE ACTIVATED SLUDGE PROCESS

The activated sludge process has been extensively modelled [41,42]. The model presented here extends existing theory by including the effects of product formation.

3.1 BATCH CULTURE

The observed production rate of microorganisms depends on the growth of new cells and auto-oxidation of existing cells. This can be written as

$$\frac{dX}{dt} = \left(\frac{dX}{dt}\right)_g + \left(\frac{dX}{dt}\right)_e \qquad (3.1)$$

where $\frac{dX}{dt}$ = observed rate of cell production.

$\left(\frac{dX}{dt}\right)_g$ = rate of new cell growth.

$\left(\frac{dX}{dt}\right)_e$ = rate of change of cell concentration by endogenous metabolism (auto-oxidation).

The rate of new cell growth is usually described by

$$\left(\frac{dX}{dt}\right)_g = \mu X = r_x^g \qquad (3.2)$$

where X = concentration of microorganisms

t = time

μ = specific growth rate.

Monod [1] showed that μ depends on the concentration of the growth limiting substrate, and he described the relationship with the hyperbolic function

$$\mu = \frac{\mu_m S}{K_s + S} \qquad (3.3)$$

where μ_m = maximum specific growth rate

S = concentration of growth limiting substrate

K_s = saturation constant, numerically equal to the substrate concentration at $\mu = \mu_m/2$.

Microorganism decay (endogenous metabolism) is usually assumed to be proportional to cell concentration:

$$\left(\frac{dX}{dt}\right)_e = -bX = r_x^e \qquad (3.4)$$

where b = specific decay rate.

It follows that Equation (3.1) can be expressed as

$$\frac{dX}{dt} = (\mu - b)X \qquad (3.5)$$

Substrate is consumed when cells grow. A yield coefficient, Y, is used to relate the amount of substrate utilized to new cells produced:

$$\left(\frac{dX}{dt}\right)_g = -Y\frac{dS}{dt} \qquad \text{i.e } r_x^g = Yr_s^g \qquad (3.6)$$

where $-\frac{dS}{dt}$ = rate of substrate consumption for synthesis of new cells.

Y = yield coefficient.

Substitution of Equations (3.6) and (3.4) into (3.1) gives the relationship between the observed cell production and substrate utilization:

$$\frac{dX}{dt} = -Y\frac{dS}{dt} - bX \qquad (3.7)$$

As regards product formation, it is proposed in this model to use the equation of Luedeking and Piret [33] (Equation (2.1)):

$$\frac{dP}{dt} = \alpha\left(\frac{dX}{dt}\right)_g + \beta X = r_p^g + r_p^e \qquad (3.8)$$

where $\frac{dP}{dt}$ = rate of product formation

α, β = constants (see section 2.3).

The above equations give the change in cell, substrate and product concentrations in batch cultures.

3.2 CONTINUOUS CULTURE

A mathematical model of the activated sludge process can be developed by applying material balances and using the relationships presented above.

A completely mixed, continuous flow, activated sludge reactor with cell recycle is shown schematically in Figure 3.1. Material balances over the reactor for cell, substrate and product are:

Cell Material Balance

Rate of change of cell concentration = Inflow - Outflow + Production due to new cell growth - Consumption due to endogenous metabolism

$$\text{i.e. } V\frac{dX}{dt} = QX_r - (GX + WX) + V\, r_X^g - V\, r_X^e \qquad (3.9)$$

Substrate Material Balance

Rate of change of substrate concentration = Inflow - Outflow - Consumption due to new cell growth

$$\text{i.e. } V\frac{dS}{dt} = (FS_o + QS) - (GS + WS) - V\, r_s^g \qquad (3.10)$$

Product Material Balance

Rate of change of product concentration = Inflow - Outflow + Production due to cell auto-oxidation + Production due to by-products of growth

$$\text{i.e. } V\frac{dP}{dt} = QP - (G + W)P + V\, r_P^e + V\, r_P^g \qquad (3.11)$$

Equations of cell, substrate and product concentrations at steady state can be obtained as follows:

The hydraulic retention time in the reactor (R_H) and sludge age (R_s) are defined by

$$R_H = \frac{V}{F} \qquad (3.12)$$

$$R_s = \frac{\text{Mass of cells in reactor and separator}}{\text{Total mass of cells wasted per unit time}}$$

$$= \frac{VX}{WX+EX_e} \qquad (3.13)$$

A flow balance over the reactor and a cell material balance over the separator give

$$F + Q = G + W \qquad (3.14)$$

$$GX = EX_e + QX_R \qquad (3.15)$$

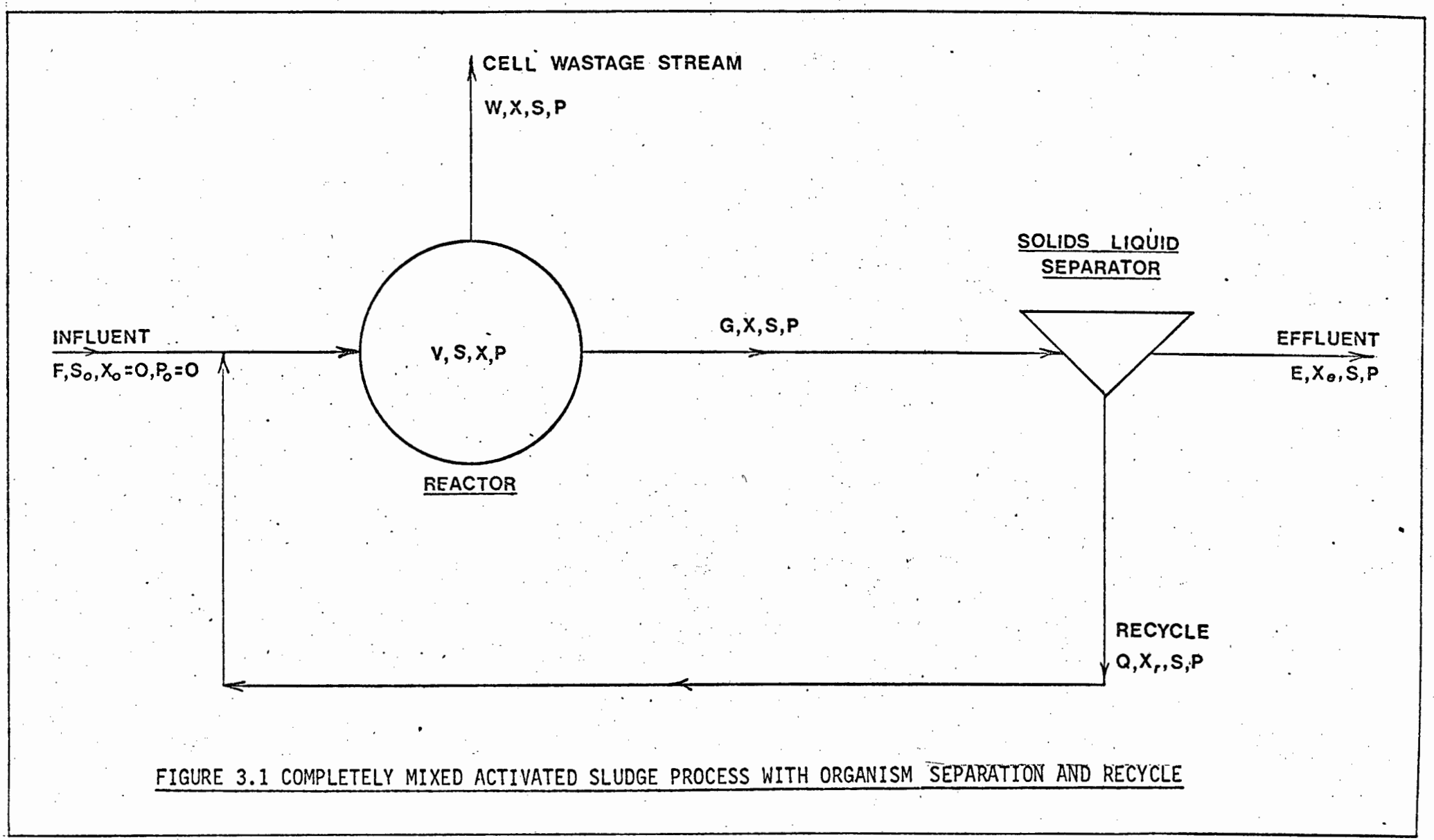


FIGURE 3.1 COMPLETELY MIXED ACTIVATED SLUDGE PROCESS WITH ORGANISM SEPARATION AND RECYCLE

Substitution of these equations into Equations (3.9), (3.10) and (3.11) gives, at steady state,

$$0 = -\frac{X}{R_S} + r_X^g - r_X^e \qquad (3.16)$$

$$0 = \frac{S_o - S}{R_H} - r_S^g \qquad (3.17)$$

$$0 = -\frac{P}{R_H} + r_p^e + r_p^r \qquad (3.18)$$

The rate terms for growth and endogenous metabolism are given in the section on batch cultures. These are summarised below.

From Equation (3.2), $r_X^g = \mu X$ (3.19)

From Equation (3.6), $r_S^g = \frac{\mu X}{Y}$ (3.20)

From Equation (3.4), $r_X^e = bX$ (3.21)

From Equation (3.8), $r_P^e = \beta X$ (3.22)

From Equation (3.8), $r_p^g = \alpha \mu X$ (3.23)

Substitution of these equations into Equations (3.17) and (3.18) gives expressions for the steady state cell and product concentrations:

$$X = \frac{Y(S_o - S)}{\mu R_H} \qquad (3.24)$$

$$P = R_H X(\beta + \alpha \mu) \qquad (3.25)$$

An expression for the specific growth rate, μ, is obtained by substituting Equations (3.19) and (3.21) into (3.16).

$$\mu = \frac{1}{R_S} + b \qquad (3.26)$$

It follows that

$$X = \frac{Y(S_o - S)}{(1+bR_S)} \cdot \frac{R_S}{R_H} \qquad (3.27)$$

The steady state substrate concentration in the reactor is obtained by combining Equation (3.26) and the Monod equation (3.3):

$$\mu = \frac{1}{R_s} + b = \frac{\mu_m S}{K_s + S} \qquad (3.28)$$

$$\therefore S = \frac{K_s(1 + bR_s)}{\mu_m R_s - (1 + bR_s)} \qquad (3.29)$$

Equation (3.29) shows that S is a function of the sludge age and the kinetic constants only.

CHEMICAL OXYGEN DEMAND (COD):

In practice it is usually difficult to measure S and P separately, and COD is often used as a measure of soluble organic carbon in solution. It is postulated that

$$COD = P + S \qquad (3.30)$$

To obtain an expression for COD in the effluent from an activated sludge reactor, it is noted that at long sludge ages the substrate concentration becomes small relative to K_s, and the Monod Equation (3.2) reduces to

$$\mu = C_1 S \qquad (3.31)$$

$$\text{where } C_1 = \frac{\mu_m}{K_s} \qquad (3.32)$$

This simplification is usually valid for the activated sludge process [43] and is generally adopted for the development of a process model.

An expression for P is obtained by substituting Equation (3.24) into (3.25):

$$P = \alpha Y(S_o - S) + \frac{\beta Y(S_o - S)}{\mu} \qquad (3.33)$$

Substitution in Equation (3.30) and simplification gives

$$COD = S\left(1 - \alpha Y - \frac{\beta Y}{\mu}\right) + \alpha Y S_o + \frac{\beta Y S_o}{\mu} \qquad (3.34)$$

As $S = \frac{\mu}{C_1}$ from Equation (3.31), it follows that

$$COD = f\mu + g + \frac{h}{\mu} \qquad (3.35)$$

where $f = \frac{1}{C_1}(1 - \alpha' Y)$ (3.36)

$$g = \alpha' Y S_o - \frac{\beta' Y}{C_1} \qquad (3.37)$$

$$h = \beta' Y S_o \qquad (3.38)$$

The model of the activated sludge process presented enables steady state cell, substrate, product and COD concentrations to be predicted. Predicted concentrations are given in Figure 3.2 for parameter values chosen from the literature. From the figure it can be seen the theory predicts that an optimum growth rate, μ_{opt}, exists for achieving minimum effluent COD. At smaller μ (longer sludge ages) the effluent COD increases due to product formation; at larger μ (shorter sludge ages) the effluent COD increases due to an increased concentration of degradable substrate.

As discussed in Section 2.3, Luedeking and Piret [33] have shown that products can be formed as a result of both cell growth and cell auto-oxidation ("growth associated and "non-growth associated" product formation respectively). In Figure 3.3 it can be seen that the auto-oxidation products (non-growth associated) are significant at low μ (long sludge age), while at shorter sludge ages the total product concentration is mainly due to the growth associated contribution.

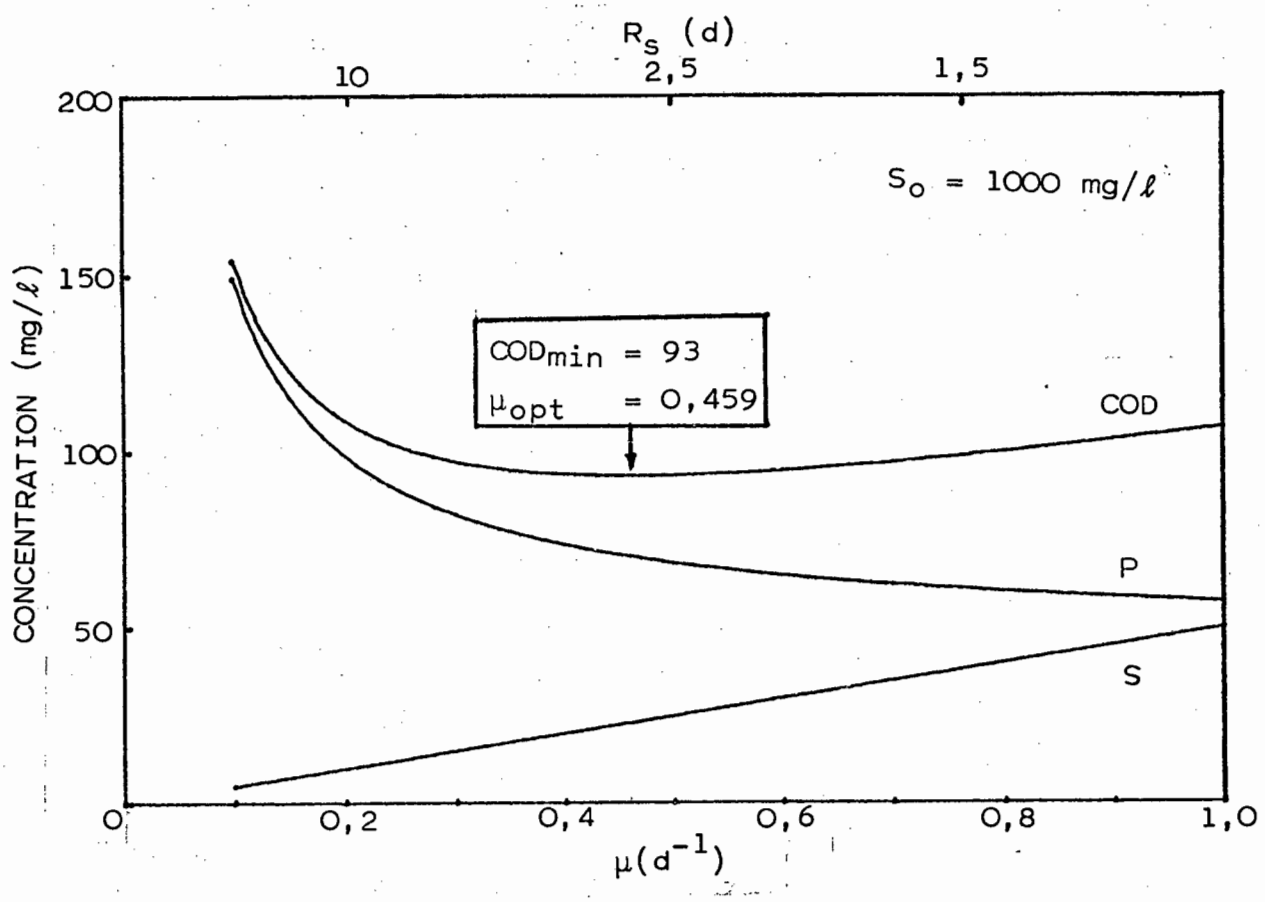
An examination of Equations (3.35) - (3.38) shows that COD is dependent upon feed strength, and a different set of curves will be obtained for each feed concentration (see Figure 3.4).

The parameters in the model depend upon the organisms and substrates used. Once the parameters for a particular combination of organism and substrate have been determined, the behaviour of the activated sludge process under varying operating conditions may be predicted.

In the following chapters, the validity of the model is tested by experiment and by the data of other workers.

Figure 3.2: Predicted substrate, product and COD concentrations for chosen values of the parameters.

Parameter Value	Source
$Y = 0,5$ mg cells/mg COD	Table 2.1
$b = 0,1\ d^{-1}$	Table 2.1
$\alpha = 0,1$	This study and Luedeking and Piret [33]
$\beta = 0,02\ d^{-1}$	This study and Luedeking and Piret [33]
$C_1 = 0,02\ \ell\ mg^{-1} d^{-1}$	Marais [43]



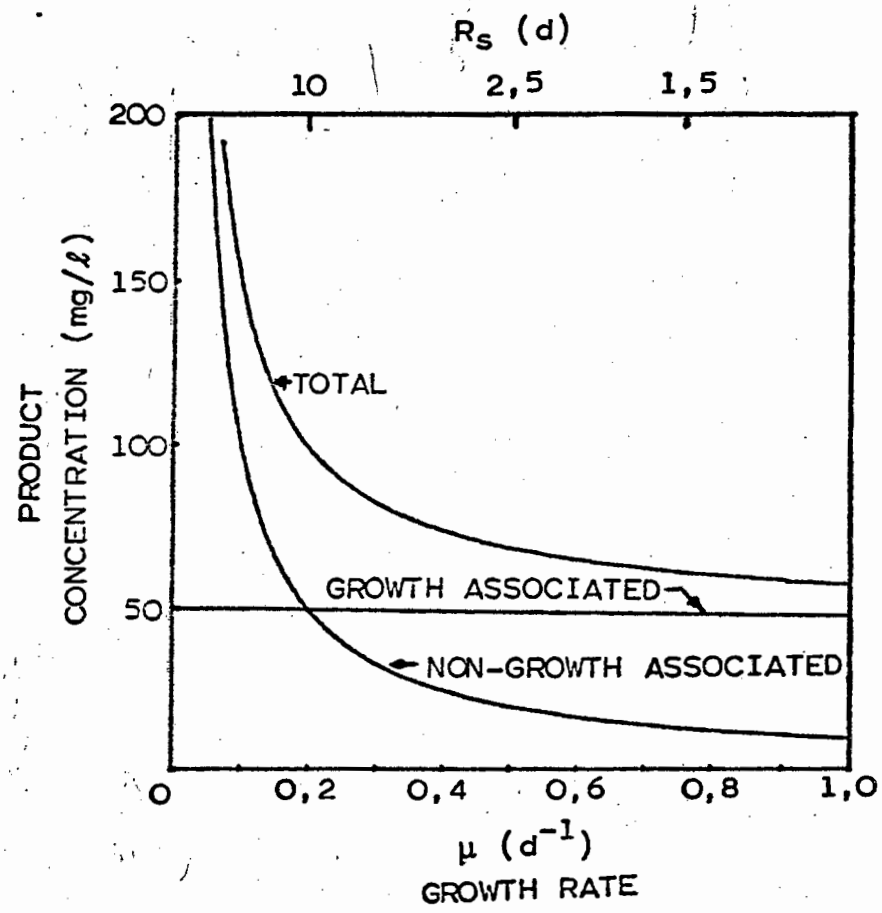
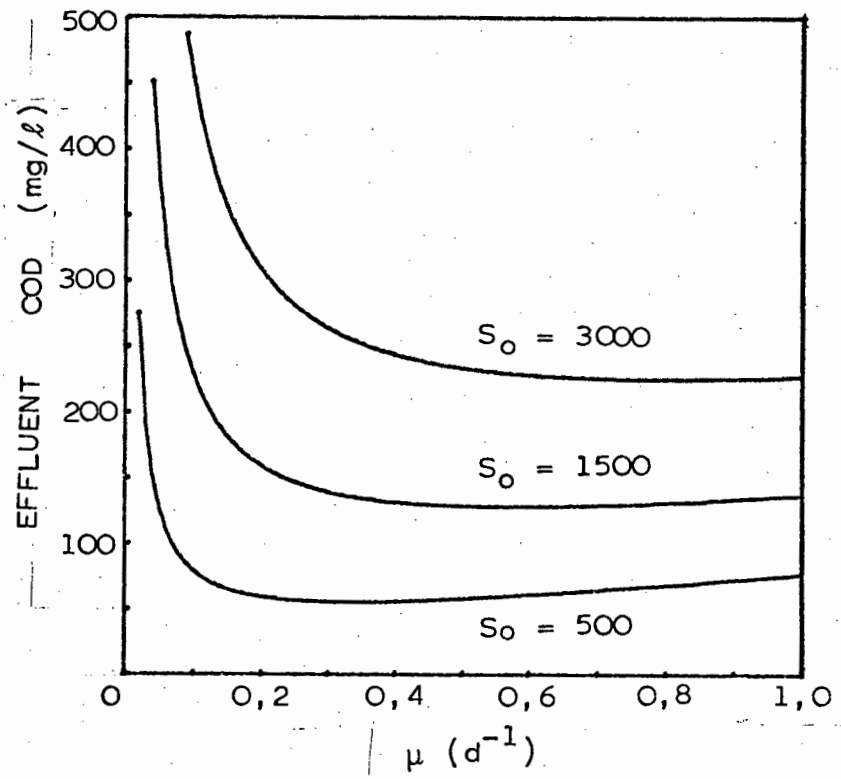


Figure 3.3. Contribution to product concentration by growth and non-growth associated mechanisms of product formation (Parameters as in Figure 3.2).

Figure 3.4 : Predicted effect of feed concentration on effluent COD concentration (Parameters as in Figure 3.2).



CHAPTER 4

EXPERIMENTAL APPARATUS AND PROCEDURES

4.1 INTRODUCTION

The growth characteristics of several substrates (shown in Table 4.1) were determined in batch culture using acclimatised populations. Two of these substrates were chosen and investigated in continuous culture. The apparatus and procedure used are described in this chapter.

Table 4.1

SUBSTRATES USED IN BATCH EXPERIMENTS

SUBSTRATE	SPECIFICATIONS	SUPPLIER	COD (mg) of 1000 mg of substrate
Manucol (Sodium Alginate)	Manucol SS/LH	ICI(SA)Ltd	600
Pectin	Rapid Set	Industrial Supp. Co(Pty)Ltd	1000
Starch	Maize Starch	Glucose & Starch Products Ltd	500
Sugar	Refined	Huletts Sugar Ltd	1099
Sewage	Settled Sewage	Athlone Sewage Works, Cape Town	COD = 600 - 900 mg/ℓ

4.2 EXPERIMENTAL APPARATUS

4.2.1 Activated Sludge Tank

The laboratory scale activated sludge unit used in this investigation was based on a design by Marais [43] and is shown in Figure 4.1. The units were constructed of clear Plexiglass so that the contents could be observed. The aerator was a porous stone bar of the type used in fish tanks. This produced fine air bubbles which provided good oxygen transfer and mixing.

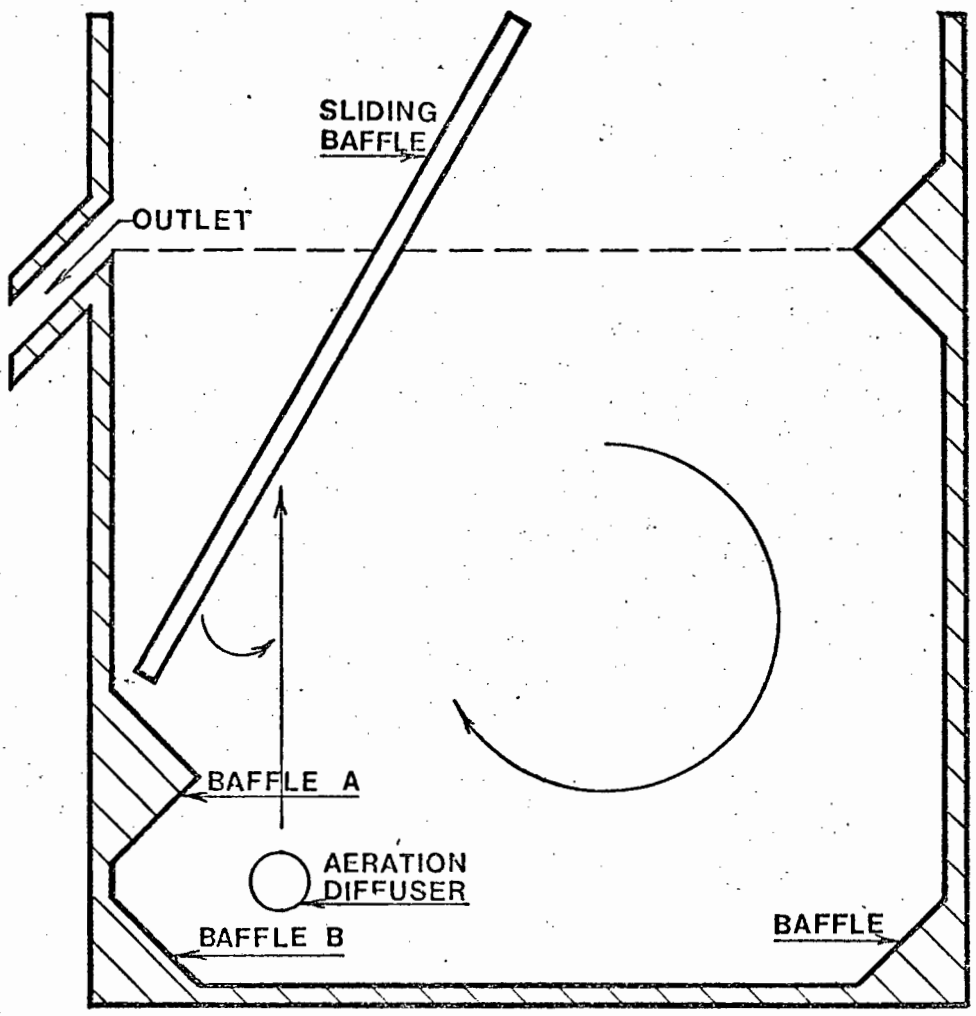


FIGURE 4.1 LABORATORY SCALE ACTIVATED SLUDGE UNIT

The optimum position for the aerator bar was that shown in Figure 4.1. The bubbles travelled up against the sliding baffle and generated a circular movement in the tank. The other baffles shown in the figure aided in eliminating dead spots in the reactor. The air bubbles deflected from the sliding baffle created eddies which drew the sludge out of the settling section back into the tank. The baffle A was added to exclude air from the settling section. In some experiments, the baffle was removed and the tanks were operated with the hydraulic residence time equal to the sludge age. This operation has been designated "flow-through" in this report.

4.2.2 Aeration and Temperature Control

The aeration system provided oxygen and mixing to the reactor and is shown in Figure 4.2. The purpose of the humidifier was to reduce evaporation losses from the reactor. Air entered the humidifier where it was intimately contacted with water maintained about 1°C above room temperature, which was maintained at 20°C by means of an air conditioning unit. The humidified air was allowed to reach this temperature in a copper tube running the length of the room. Any water that condensed was stripped out, and the humidified air was passed through a rotameter before entering the tanks. The resultant loss of water from the tanks due to evaporation was small, and the temperature in the tanks could be readily maintained at 20°C.

4.2.3 Lighting

The constant temperature room had fluorescent lighting. In order to keep the environment as constant as possible, the lights were kept on at all times.

4.2.4 Feeding Apparatus

The system that was designed to feed the tanks is shown in Figure 4.3. A stoppered 25-litre polythene feed container was fitted with a capillary tube containing a length of close fitting wire. When liquid flowed out of the feed container

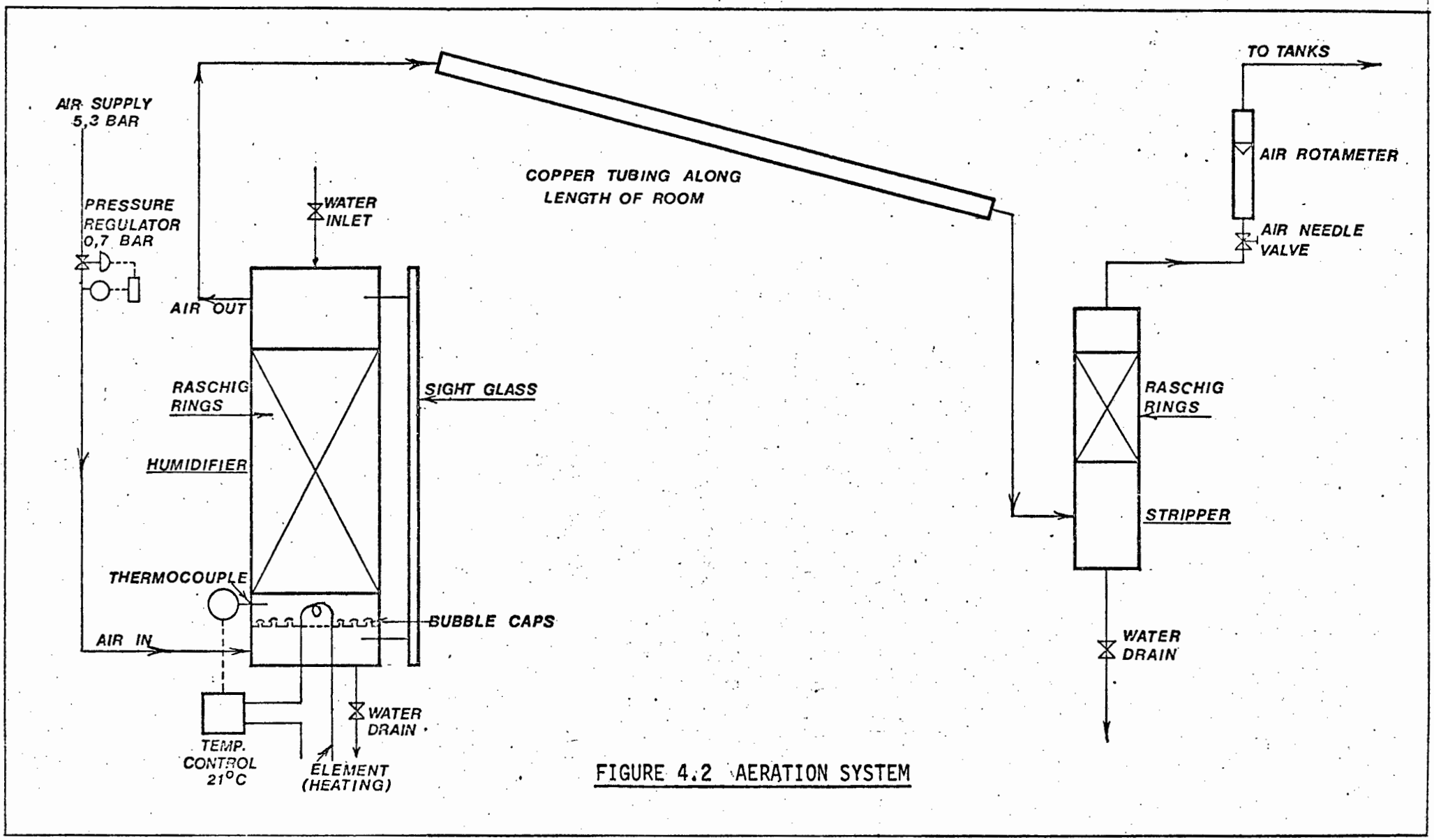


FIGURE 4.2 AERATION SYSTEM

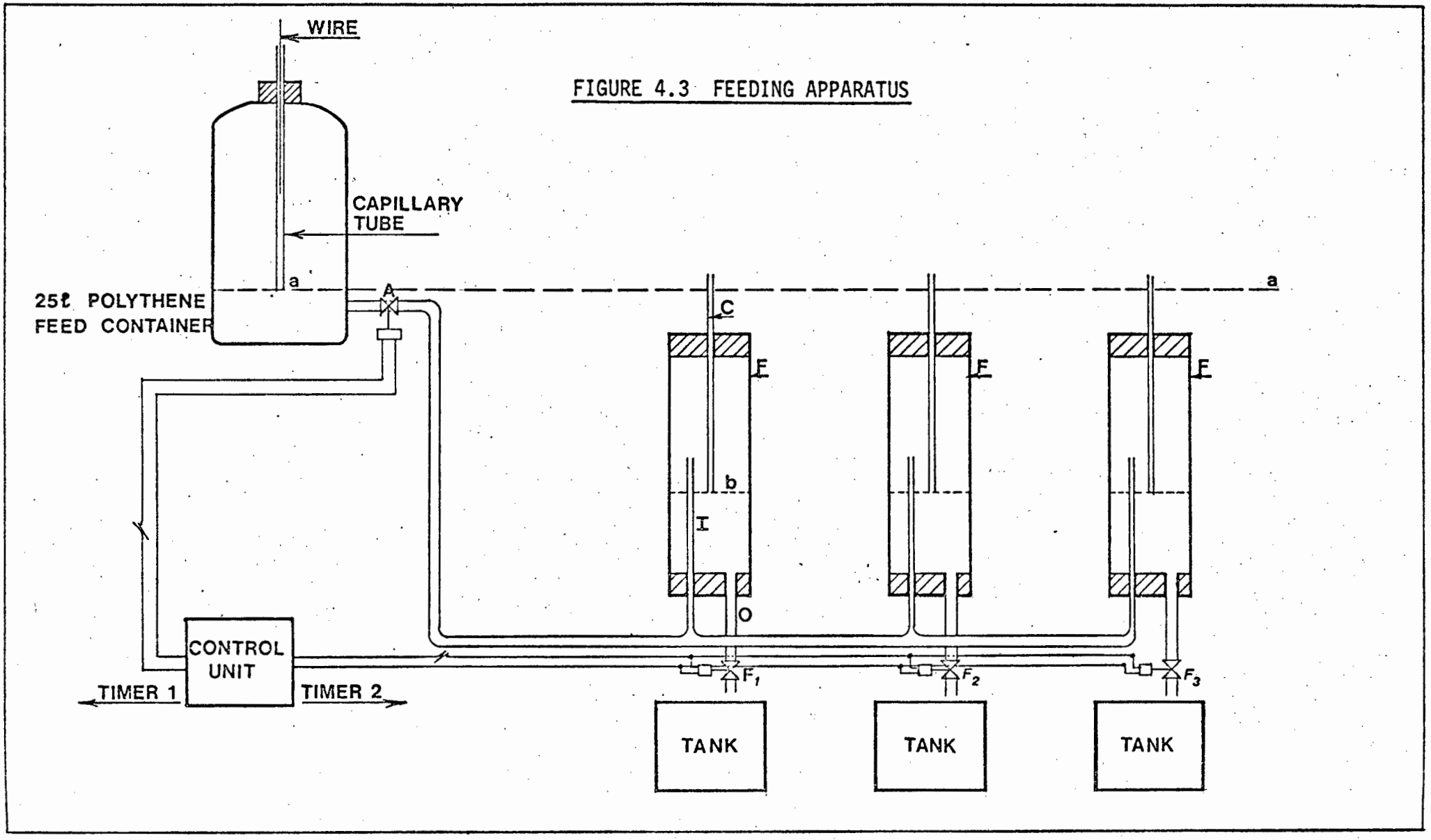


FIGURE 4.3 FEEDING APPARATUS

through A, air was drawn in through the capillary tube and the pressure at a was always atmospheric. The rate of flow through valve A was adjusted by altering the length of the wire in the capillary. The feeders F consisted of glass tubes stoppered at both ends. The top of the feeder was fitted with a capillary tube C. The bottom had an inlet tube I and an outlet tube O. A, F_1, F_2 and F_3 were solenoid valves (Asco $^1/_4$", Automatic Switch Co). These were activated by a control unit which consisted of two timers (Omron, Type STP-NM2) and a relay (Omron, Type LY-4).

Timer 1 activated valve A, and Timer 2 activated valves F_1, F_2 and F_3. When Timer 1 was on, valve A was open and the feed flowed from the feed container into the feeders F. Valves F_1, F_2 and F_3 were closed during this time, and the liquid filled the feeders until the pressure was equalised at a. The volume of liquid in the capillary tubes C was negligible and the volume of liquid to be delivered to the tanks could be adjusted by moving C to change the level b.

After the set period, Timer 1 switched off and activated Timer 2. Valve A then closed, preventing any more liquid from flowing into the feeders. Valves F_1, F_2 and F_3 opened allowing the liquid in the feeders to flow into the tanks. This process was repeated continuously, and the tanks were fed with a fixed volume of liquid at regular and frequent intervals.

Each tank was fed by two feeders, one feeding the substrate and the other the nutrient solution. In this way growth in the feed lines was minimised. The substrate and nutrient were always delivered to the tanks in equal volumes, and the two streams were fed simultaneously. All valves on the feeders were operated by one control unit. A schematic diagram of the feeding system arrangement in shown in Figure 4.4.

Frequent checks were made to ensure that the volumes delivered by the apparatus were consistent. The apparatus was designed for easy cleaning and periodically the system was dismantled and cleaned in a solution of sodium hypochlorite and then hot water. The valves also needed periodic maintenance and were easily dismantled. The feed containers were replaced

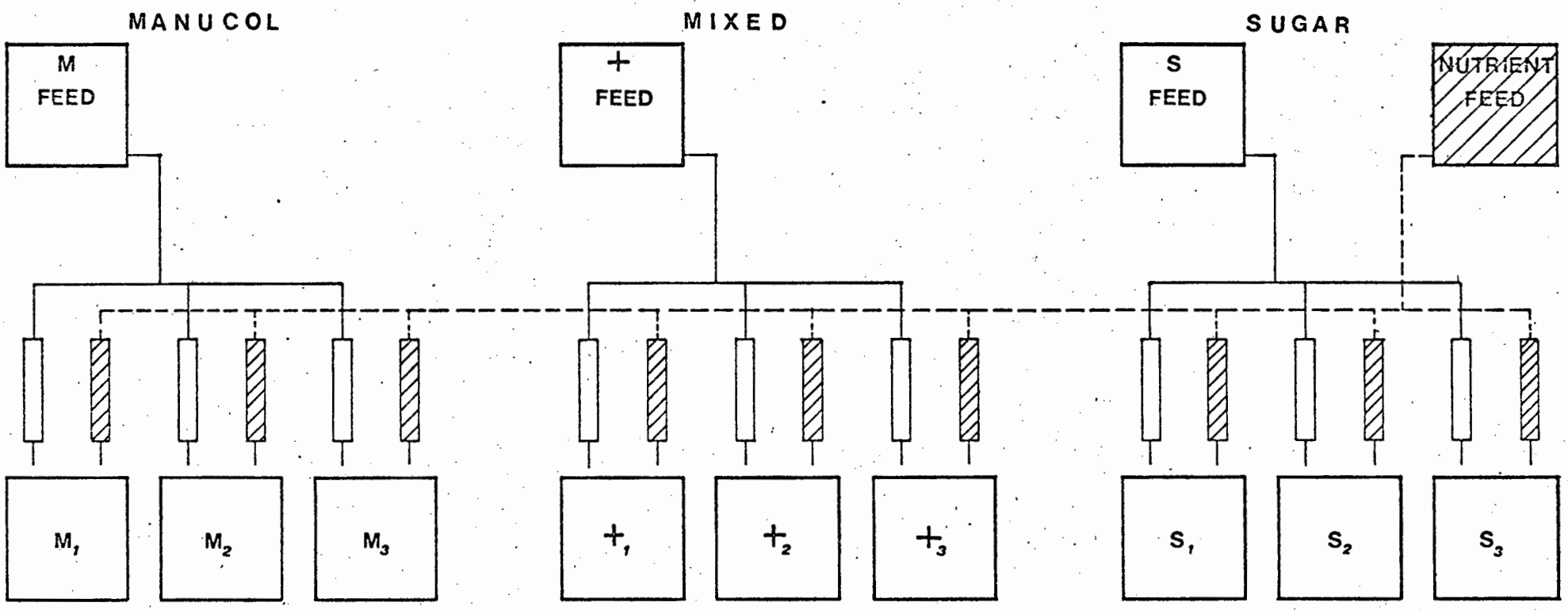


FIGURE 4.4 FEEDING SYSTEM ARRANGEMENT

with clean drums which had been allowed to soak in the hypochlorite solution, and then hot water, after each 25 litres of feed.

4.2.4.1 Feed and Nutrient Make-Up: Fresh feed was made up every second day. Sugar dissolved readily in distilled water but a high speed stirrer was required to dissolve manucol. The stirrer was placed in distilled water and manucol powder was sprinkled into the vortex. If this was not done, the powder formed coagulated lumps which were very difficult to dissolve. The solution was stirred for one to two hours before use. For mixed substrate experiments, the feed always consisted of 50% by COD of manucol and 50% by COD of sugar.

The nutrient solution was made up in concentrated form and diluted as required. The composition of the solution is given in Table 4.2. These concentrations have been used by several workers [18, 26] and satisfied the requirements of a BOD:N:P ratio of 100:5:1 [44]. The nutrients provided a phosphate buffer. Activated sludge is little affected by pH changes in the range 6 - 9, but the optimum is pH 7 - 7,5 for the activated sludge treatment of domestic waste water [45]. Chiu et al. [18], using the concentrations given in Table 4.2, were able to maintain their pH at 6,5 - 7,1.

The phosphate buffer was kept separate from the rest of the nutrient solution until required in order to prevent the precipitation of any phosphate salts. The concentrated stocks were made up such that 1 litre of the nutrient solution plus 60 mℓ of buffer plus 9 litres of water (tap and distilled water, accoding to the concentrations given in Table 4.2) gave 10 litres of nutrients which was sufficient for a substrate concentration of 2 000 COD. As the feed and nutrient streams were delivered in equal volumes, the feed COD of 2 000 mg/ℓ was diluted by the nutrient solution to give a feed of 1 000 mg/ℓ COD, plus all the nutrients required for balanced growth at this concentration.

TABLE 4.2

COMPOSITION OF GROWTH MEDIUM

Constituent	Concentration (mg/ℓ)
Carbon Source	1 000 COD
$(NH_4)_2SO_4$	500
$MgSO_4 \cdot 7H_2O$	100
$FeCl_3 \cdot 6H_2O$	0,5
$MnSO_4 \cdot H_2O$	10,0
$CaCl_2$	7,5
KH_2PO_4	527
K_2HPO_4	1 070
Tap Water	100 mℓ/ℓ
Distilled water	To volume

4.2.5 Siphons

When the tanks were operated as flow-through systems, siphons were incorporated at the tank exit to prevent filtering of the solids at the exit pipe. Several different designs of siphons were investigated. These are further discussed in Appendix D.

4.3 EXPERIMENTAL PROCEDURES

4.3.1 Acclimatisation

Microbial populations were developed on each carbon source according to the following procedure: 1-litre conical flasks containing 800 mℓ of raw sewage were aerated through glass diffusers at approximately 1,5 ℓ of air per minute. This was seeded with microorganisms obtained from a laboratory activated sludge unit operating at a sludge age of 10 days and a hydraulic age of 1 day, on raw sewage obtained from the Athlone Sewage Works, Cape Town.

Approximately every 12 hours 40 mℓ of the MLSS was discarded from each flask. The air was switched off for about 1 hour to allow the solids to settle, after which 360 mℓ of the supernatant was discarded. 400 mℓ of fresh feed was then added. The flasks were thus operated at a sludge age of 10 days and a hydraulic age of 1 day.

Solutions of the substrates were made up to the same COD strength (600 mg/ℓ) as the raw sewage according to the concentration relationships shown in Table 4.1. Each carbon source was provided with the nutrients required for growth according to the concentrations given in Table 4.2.

The feed to each acclimatisation flask consisted of sewage and the substrate solution to which microorganisms were to be acclimatised. The substrate fraction of the sewage-substrate feed was progressively increased and the COD and cell concentration parameters were used to determine the course of the acclimatisation. The results are discussed in Chapter 5.

When the flasks were able to degrade a feed of pure substrate, the COD of the feed was increased to 1000 COD. The procedure that was then adopted was to discard 2/3 of the stock from each flask and replenish with fresh medium. When growth on the fresh substrate was complete, as shown by increased flocs of microorganisms in the tank, the procedure was repeated. This occurred about every 2 days.

This procedure was continued for about 8 weeks, after which it was considered that the microorganisms were fully acclimatised to the substrate they were being fed on.

4.3.2. Batch Experiments

Before a batch test was started the cell concentration in the acclimatisation flask was allowed to build up by not discarding any of the cells. An accurate measurement of the cell concentration was then determined gravimetrically. The innoculum concentration to each batch tank was calculated by transferring a known volume of MLSS to the batch tank.

Five litres of each carbon source at 1000 mg/ℓ COD, together with the required nutrient (Table 4.2), were placed in the batch tanks. These were the 6-litre vessels used for the continuous culture experiments, with the baffles removed (Figure 4.1). The aerator bar was used to provide oxygen transfer and mixing. The tanks were innoculated from the acclimatisation flasks, and the COD, cell concentration,

temperature, pH and dissolved oxygen were recorded. It is noted that the batch experiments were not conducted in the constant temperature room used for the continuous culture experiments.

4.3.3 Continuous Culture Experiments

Experiments were done using the apparatus shown in Figure 4.1. Humidified air at 20°C was used to supply oxygen and mixing to each tank. Feed and nutrients were fed in separate lines using the automatic feeding device. The tanks were innoculated with seed from the acclimatisation flasks.

The COD and cell concentrations were monitored regularly until a steady state was obtained at each experimental condition. A careful check was kept on temperature, pH and dissolved oxygen concentration. Spot checks were done on the BOD and nutrient concentrations in the effluent.

For some experiments the tanks were operated with settling compartments for the cells. The sludge age was set by removing the baffle and withdrawing the required volume of MLSS. This was usually done every 12 hours. In other experiments the baffle was removed to provide flow-through operation.

4.4 MAINTENANCE

The maintenance procedure for the feeders and feed containers has already been described. The activated sludge tanks were kept scrupulously clean. The walls of the tanks were scraped twice a day, and periodically the tank was dismantled for cleaning with a soap solution.

4.5 ANALYTICAL TECHNIQUES

Samples were analysed for chemical oxygen demand (COD) carbohydrate concentration and biochemical oxygen demand (BOD); mixed liquor suspended solids (MLSS) and mixed liquor volatile suspended solids (MLVSS); pH, dissolved oxygen concentration, temperature, and sludge volume index (SVI).

Analyses were always done by extracting a sample from the tank itself, and not on the effluent collected (except for the effluent solids). For tanks operated with settling sections, the sliding baffles (Figure 4.1) were removed and the reactor contents were thoroughly mixed before withdrawing any sample.

A 100 mℓ sample was withdrawn from the tank at the prescribed time and centrifuged at 4000 rpm in a Super Minor Centrifuge (Measuring and Scientific Equipment Limited) for about 20 minutes. The sample was filtered through a pre-weighed filter paper under vacuum. For batch experiments, Whatman GFA No. 541 filter paper was used. For the rest of the experiments, glass fibre filter paper (Whatman GF/A) was used as this paper was not hygroscopic. The filter paper together with three preweighed blanks were allowed to dry in an oven at 105°C overnight. The papers were placed in a dessicator for several hours prior to reweighing. The papers were corrected for change in moisture content, which was very small for the glass papers.

The MLVSS were determined by placing the filter papers (plus blanks) in an oven at 590°C for 30 minutes. The samples were again allowed to stand in a desiccator for several hours prior to reweighing.

For some experiments (batch) the cell concentration was also determined by measuring the light absorbance at 510 nm wavelength in a Beckman 1211 colorimeter. Later a Hach Model 2100A Turbidimeter became available and this was used instead.

Sludge Volume Index (SVI)

Determined according to Standard Methods procedure [46].

Substrate Determination

For many of the experiments the COD was determined by the dichromate reflux method as prescribed in Standard Methods [46]. During the course of this investigation a Technicon Auto Analyzer became available and the COD determin-

ations were done on both glass fibre and millipore filtrates.

In some experiments the carbohydrate content of the effluent was estimated by the method of Dubois et al. [47] using a Beckman photometer 1211.

The BOD was determined manometrically in a Hach BOD apparatus, model 3273.

pH

The pH was measured using a Metrohm Herisan pH-Meter E520. The meter was always standardised to a pH of 7 by using a buffer solution before measuring the pH of a sample.

Dissolved Oxygen and Temperature

These were measured using an oxygen probe supplied by the Yellow Springs Instrument Co. Inc. (Model 540). The instrument was air calibrated according to the manufacturer's procedure before use. For batch experiments, dissolved oxygen was monitored continuously by connecting the oxygen meter to a Hewlett Packard Model 680 strip chart recorder.

Nutrient Concentrations

Spot checks were made on the nutrient concentrations (cations) by using a Varian Techtron Model 1100 atomic absorption spectrophotometer.

Microscopic Observations and Photographs

A Nikon Automatic Microflex Model AFM microscope fitted with a Nikon Polaroid camera attachment were used.

CHAPTER 5

RESULTS

5.1 INTRODUCTION

A natural population was grown by continuous culture on each of two substrates and a mixture of them to determine the growth characteristics on a two-substrate feed. It was advantageous for the two materials to have μ_m values an order of magnitude apart so that the influence of each substrate on the mixed feed growth curve could be readily observed. To find such substrates, several carbohydrates were examined in batch culture using acclimatised populations. The first part of Chapter 5 describes these experiments and how they led to the choice of the substrates used in continuous culture. The latter part of the chapter shows how product formation resulted in growth curves for both the single and dual substrate feeds which could not be explained by Monod's equation. A model for product formation was then formulated to explain the growth curves of the systems investigated.

5.2 ACCLIMATISATION PROCESS

Microorganisms were acclimatised to manucol, sugar, pectin, starch and sewage (see Table 4.1) according to the procedures indicated in Chapter 4. The results for manucol and sewage are shown in Figure 5.1. The volume of sewage in the feed to the manucol flask was progressively decreased and replaced with manucol solution until the flask was able to operate on a feed of pure substrate (see Figure 5.1.a).

The effluent COD in the flask operating on sewage only remained steady (Figure 5.1.b). The initial addition of manucol to a flask which had been operating on sewage did not appear to affect the feed degradation. When the manucol percentage in the feed was increased from 10 to 30, the COD in the flask increased indicating that manucol was not metabolised. After about 11 days the population adapted to the manucol feed and the COD in the flask dropped.

The sugar and starch flasks were able to degrade the substrates immediately. The pectin was initially not degraded,

but over the course of several days the microorganisms became adapted to the feed and the COD of the flask dropped to a low level.

A population that was acclimatised to each substrate was obtained and the growth kinetics of these microorganisms were examined in batch experiments.

5.3 BATCH EXPERIMENTS

The experimental results are shown in Figure 5.2 and are tabulated in Appendix A.

Typical batch culture behaviour was observed. There was an initial lag phase, followed by a logarithmic growth phase and a stationary phase. In some experiments an acceleration phase was observed. The lag phase depends on the history and the size of the inoculum; the relatively long lag phases observed here are expected because of the small inocula used [48].

Cell concentration data were used to estimate the relative degradabilities of the substrates, as follows: Substitution of Equations (3.2) and (3.4) into Equation (3.1) gave

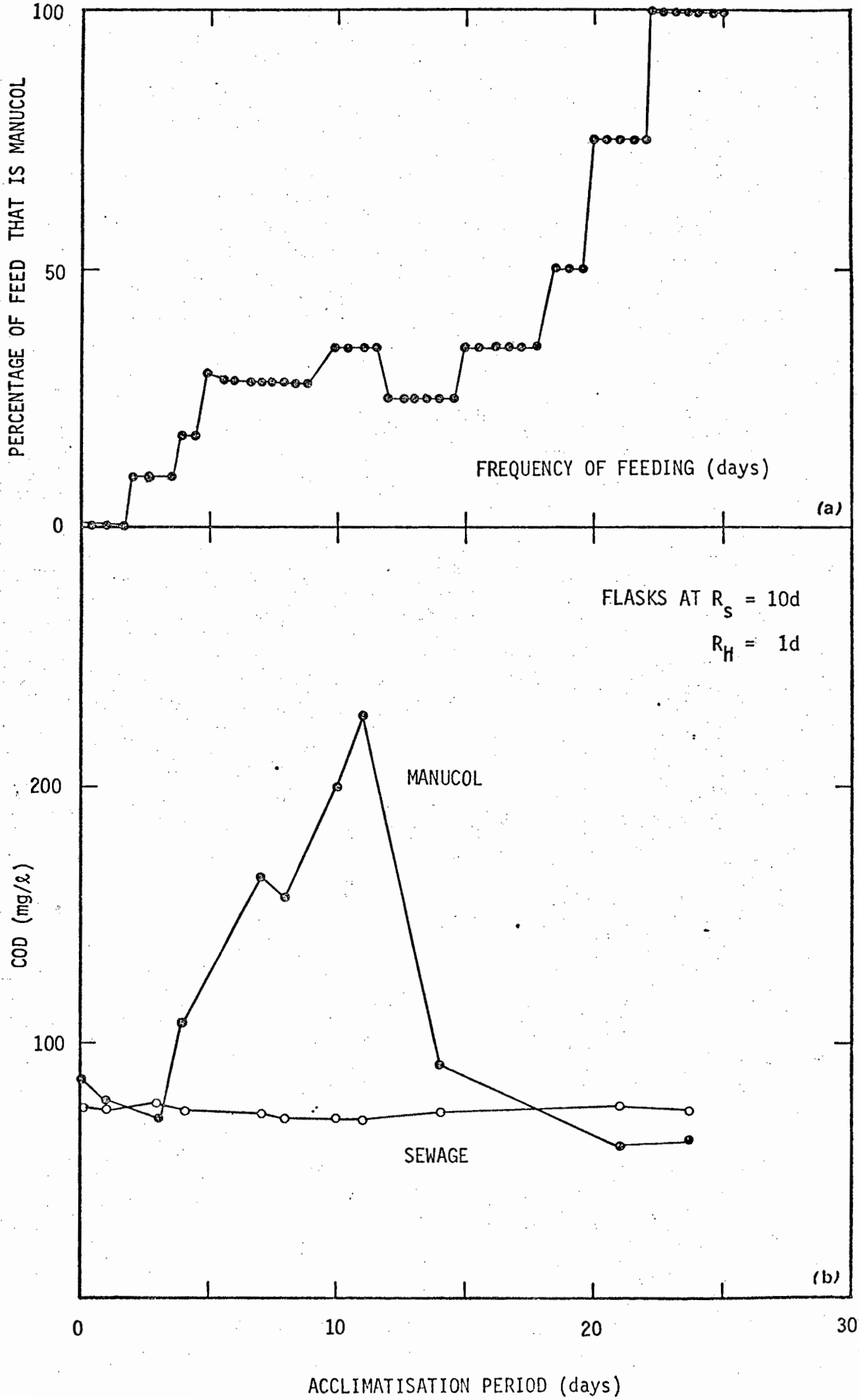
$$\frac{1}{X}\frac{dX}{dt} = (\mu - b) \qquad (5.1)$$

Then $$\frac{d}{dt}(\log_e X) = \frac{1}{X}\frac{dX}{dt} = (\mu - b) \qquad (5.2)$$

A plot of $\log_e X$ versus time should give a curve of slope $(\mu - b)$; during the logarithmic growth phase the plot should be a straight line of slope $(\mu_m - b)$. The plots are shown in Figure 5.3, and the results are summarised in Table 5.1.

The relative degradabilities of the substrates were found to be in the order sugar > pectin > starch > manucol. Sugar, pectin and starch had similar $(\mu_m - b)$ values, and these were comparable to the values obtained by Peil and Gaudy [26] for a variety of carbohydrates. The $(\mu_m - b)$ value for manucol was an order of magnitude smaller than that of sugar, and it was decided that sugar and manucol should be suitable for the continuous culture investigation.

FIGURE 5.1 ACCLIMATISATION TO MANUCOL



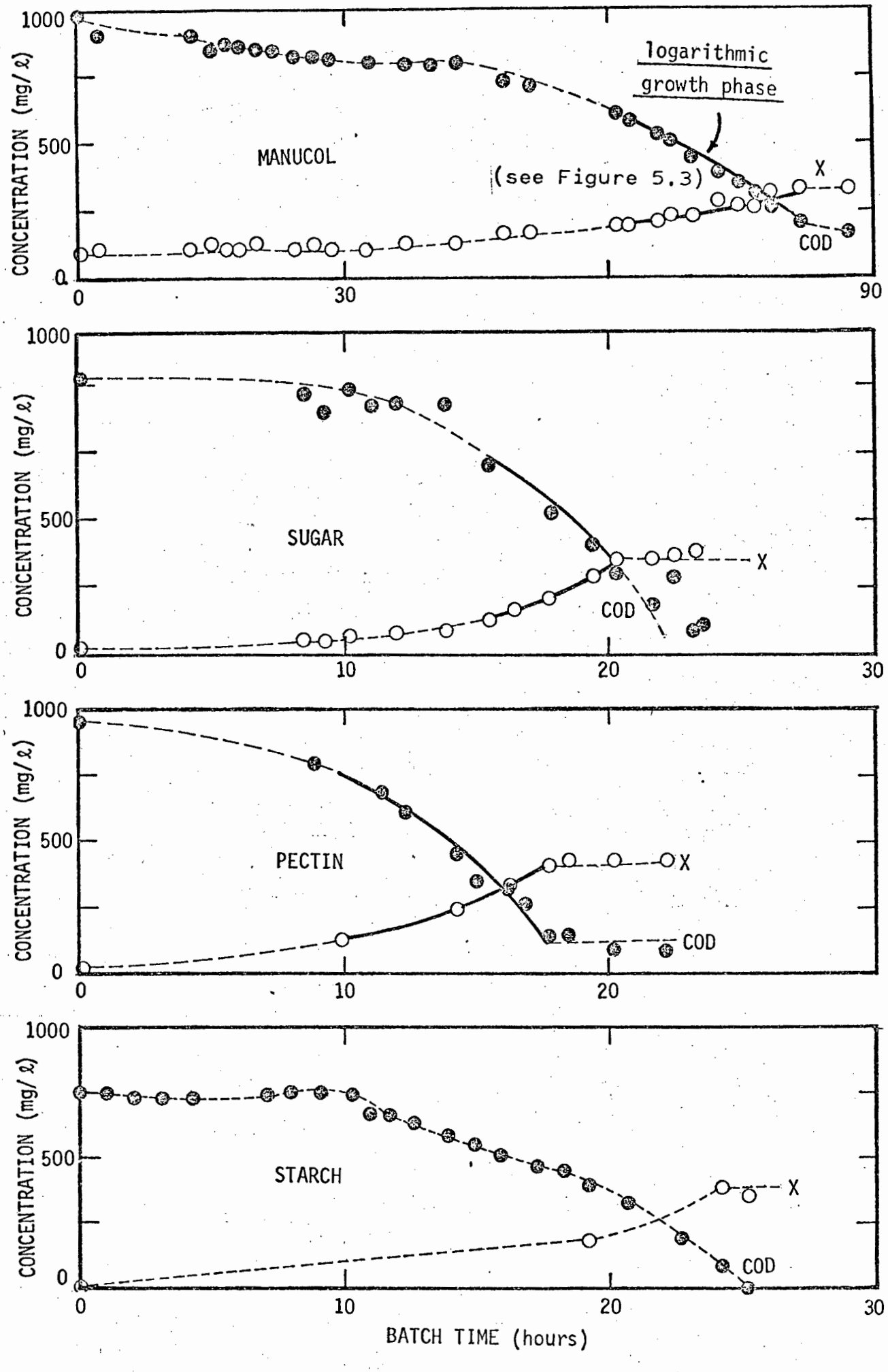


FIGURE 5.2 BATCH EXPERIMENTAL RESULTS

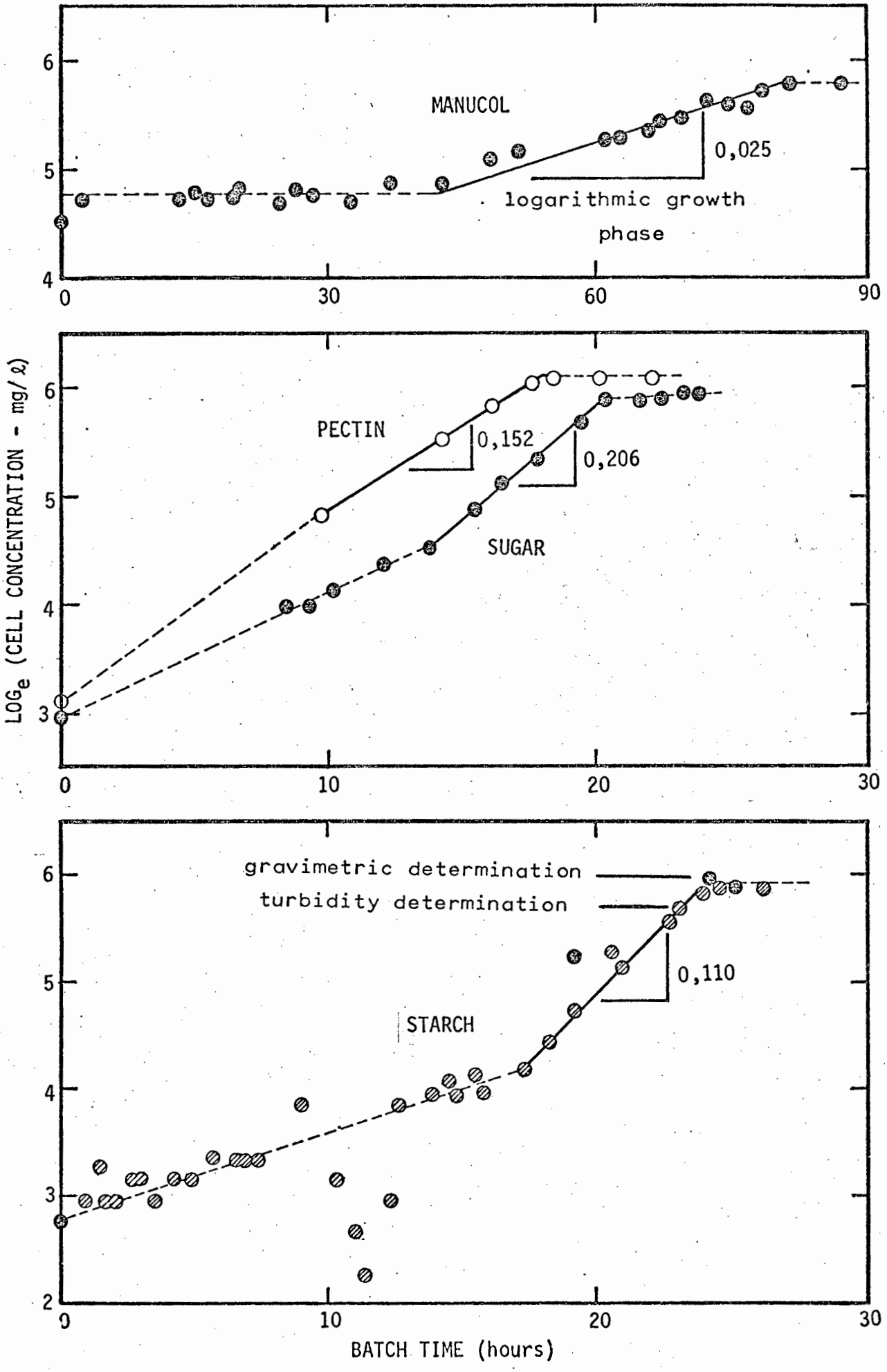


FIGURE 5.3 MAXIMUM SPECIFIC GROWTH RATE FROM BATCH EXPERIMENTS

TABLE 5.1

BATCH EXPERIMENTAL RESULTS

SUBSTRATE	S_o (mg/ℓ COD)	X_o (mg/ℓ)	Lowest COD (mg/ℓ)	Duration of Growth Phases (h)				Yoverall* solids produced /COD degraded	μ_m-b (h^{-1})
				Lag	Acceleration	Log Growth	Total		
MANUCOL	981	95	163	35	26	20	81	0,29	0,025
SUGAR	1027	20	92	14	1	6,4	21	0,39	0,206
PECTIN	950	22	91	10	0	8	18	0,48	0,152
STARCH	750	16	0	17	0	6	23	0,47	0,110

* The overal yield was calculated as follows: $Y_{overall} = \frac{\text{final solids - initial solids}}{\text{initial COD - final COD}}$

5.4 CONTINUOUS CULTURE EXPERIMENTS

The growth characteristics of a natural microbial population were measured in continuous culture on each of manucol, sugar, and a mixture of the two, and steady state COD and cell concentrations were obtained over a wide range of sludge ages. Initially experiments were done at long sludge ages; the experimental details for manucol (Tanks 1, 2, 3) and mixed substrates (Tanks 23, 24, 25) are tabulated in Appendix B. No long sludge age experiments were done on sugar as its maximum growth rate (from batch experiments) indicated that the sugar substrate concentration should be low at long sludge ages. (At this stage, it was not realised that product formation could be significant at long sludge ages.) Typical results for manucol and mixed substrate are shown graphically in Figures 5.4 and 5.5.

Parameters which could be controlled, such as feed rate and sludge withdrawal, were kept constant to allow the tanks to achieve steady state operation. Although the bacterial flow settled well - this is indicated by the low SVI values measured: for example SVI equals 50 for Tank 1 - there was a dispersed population in the liquid which did not settle at all, and this resulted in the cell concentration measured in the effluent. The effect of losing solids with the effluent was to lower the sludge age (see Equation 3.13), and this in turn affected the cell and COD concentrations in the tank. (As is expected, it can be seen from the graphs that when the sludge age was high, the cell concentration in the tank was high, and the COD was low.) The tanks were operated for 3 months in an attempt to obtain steady states. The effluent cell concentrations were seldom steady, and the other parameters varied sympathetically. There were, however, long periods during which the variation of the parameters was within a narrow range of values. The average values of the parameters for these periods are tabulated in Table 5.2.

To overcome the problem of varying overflow cell concentration, the tanks were operated as flow-through systems. The effluent cell concentration was then equal to the cell concentration in the tank and the sludge age was controlled hydraulically.

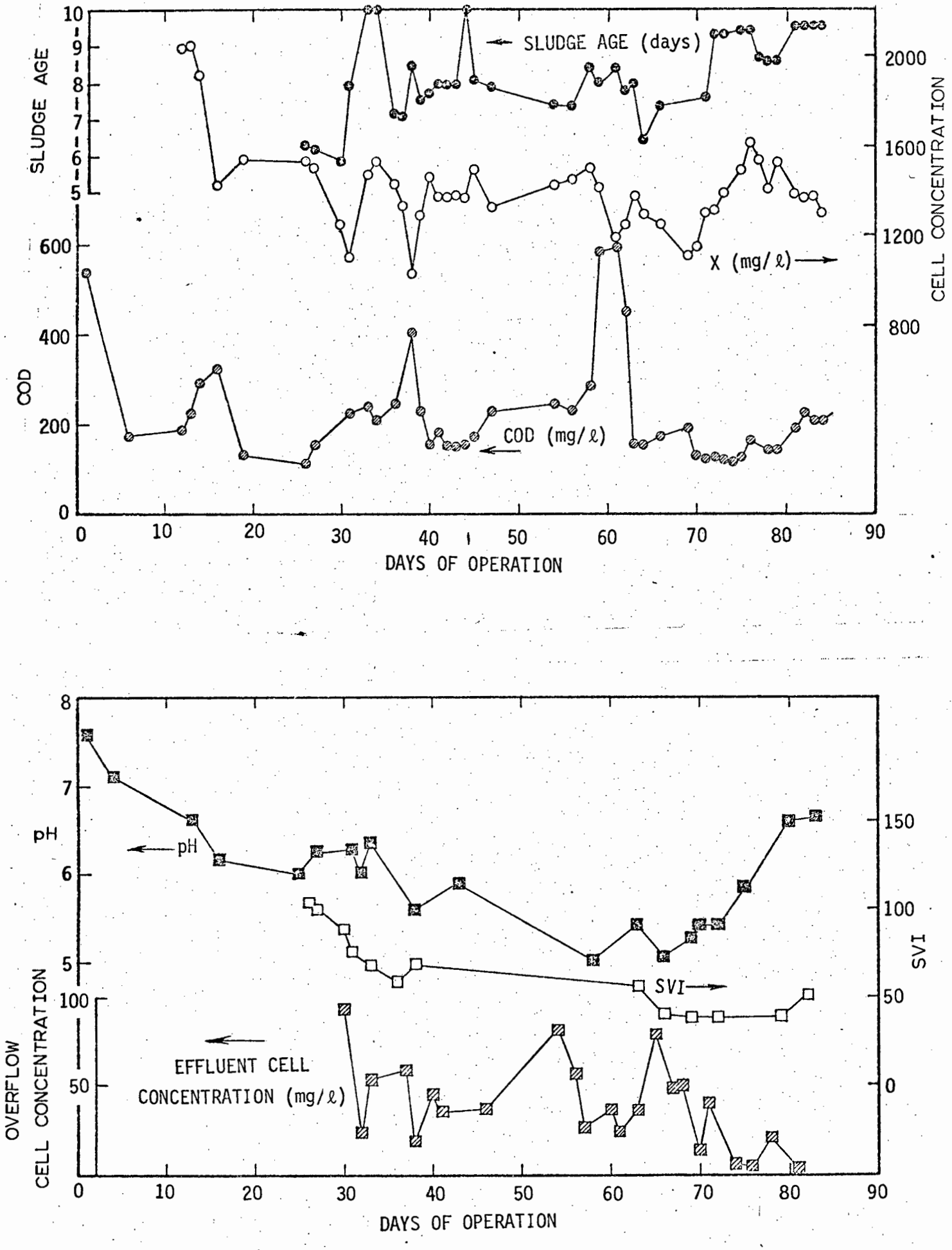


FIGURE 5.4 MANUCOL TANK NO: 1 (operated with settling compartment).

FIGURE 5.5 MIXED TANK NO: 23 (Operated with settling compartment).

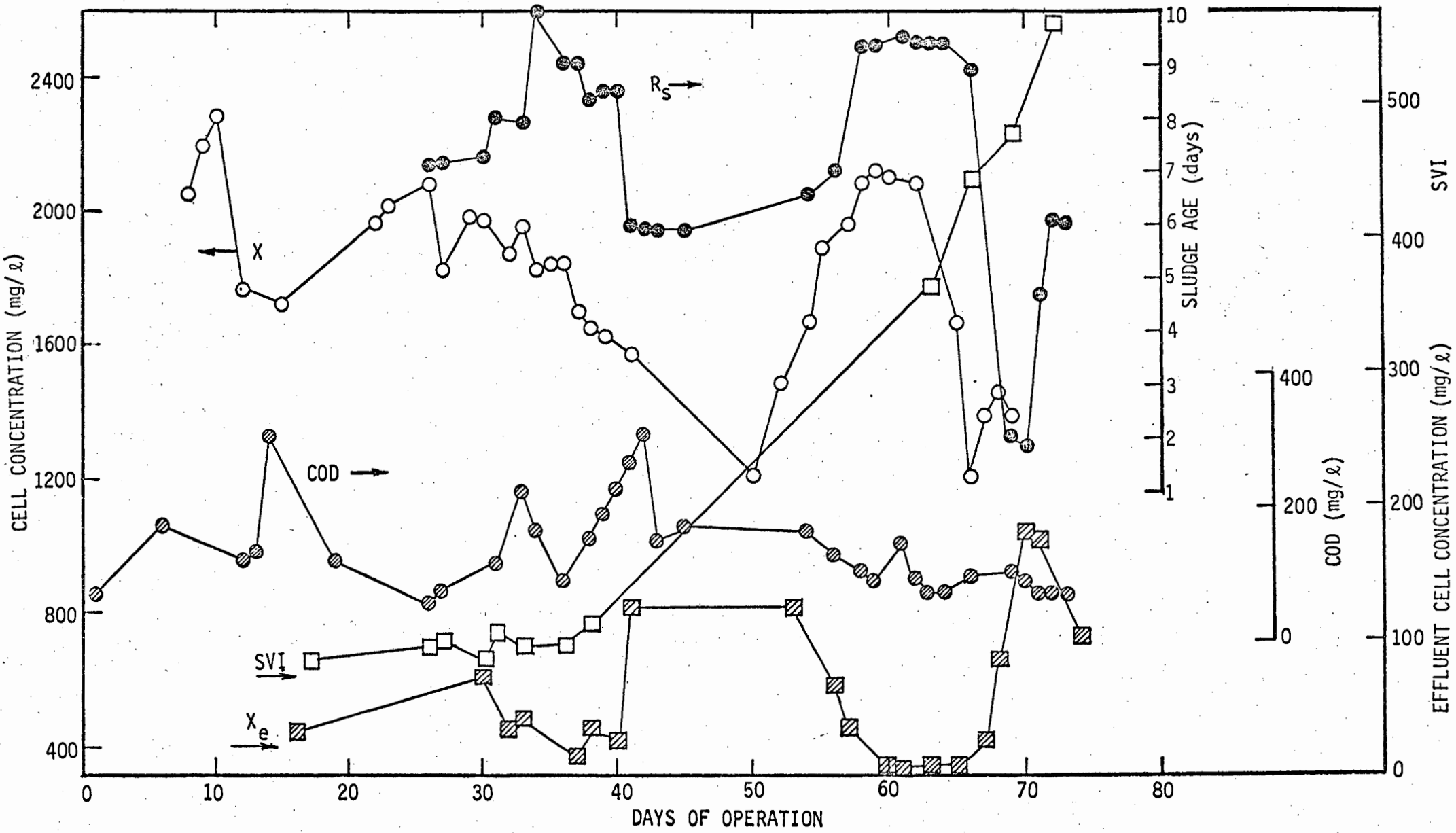


TABLE 5.2

STEADY VALUES FOR TANKS OPERATED WITH SETTLING SECTIONS

TANK NO	SUBSTRATE	PERIOD CONSIDERED (days)*	R_s d	COD mg/ℓ	X mg/ℓ	SVI
1	Manucol	31 - 58	7,76	209	1413	60
1	"	72 - 84	9,22	162	1424	40
2	"	33 - 58	9,00	148	1708	94
2	"	70 - 84	7,80	226	1093	62
3	"	36 - 59	9,10	160		93
23	Mixed	26 - 40	8,12	145	1930	97
23	"	58 - 66	9,34	97	2100	365
24	"	25 - 40	8,19	90	2048	70
24	"	70 - 82	8,46	97	1550	274
25	"	60 - 76	7,71	84	1600	223

* Refer to Appendix B.

Biolgical systems are inherently variable and, to determine whether a steady state was attainable - and if so, whether this steady state condition was reproducible - three activated sludge units were operated on each system at approximately the same sludge age (equal to hydraulic residence time). Steady state operation was generally difficult to achieve, and a long period of operation (3 months) was required to estimate average values with some degree of confidence.

Flow-through experiments on sugar at sludge ages of less than 2 days were performed, but these were not very successful as the sugar substrate resulted in a dispersed population, and it was found very difficult to separate the microorganisms from solution. This resulted in erroneous values for COD and cell concentrations, and steady state concentrations of these parameters were never recorded. It was considered that the results obtained for sugar operation were unreliable, and were discarded.

The operating conditions and results for flow through tanks operating on manucol (Tanks 4, 5, 6) and mixed substrates (Tanks 26, 27, 28) are summarised in Appendix B, and steady state results are given in Tables 5.3 and 5.4. These show that flow-through tanks operated at the same conditions of sludge age and feed strength give reproducible results.

TABLE 5.3

STEADY STATE RESULTS FOR FLOW-THROUGH TANKS OPERATING ON MANUCOL AT SIMILAR SLUDGE AGES

TANK NO.	R_s (d)	COD (mg/ℓ)	Range	X (mg/ℓ)	Range
4	4,803	381	254 - 585	1081	844 - 1262
5	4,647	331	280 - 371		600 - 1600
6	4,579	379	331 - 406	1020	740 - 1039
Average	4,676	364		1050	

TABLE 5.4

STEADY STATE RESULTS FOR FLOW-THROUGH TANKS OPERATING ON MIXED SUBSTRATES AT SIMILAR SLUDGE AGES

TANK NO.	R_s (d)	COD (mg/ℓ)	COD(millipore) (mg/ℓ)	X (mg/ℓ)
26	4,74	270	249	1076
26	4,65	218	201	988
27	4,65	228	198	761
28	4,60	187	187	881
Average	4,66	226	209	927

Flow-through tanks were operated at different residence times to generate steady state data needed to calculate the kinetic constants. The experimental procedures and results for Tanks 7 - 17 (manucol) and Tanks 29 - 37 (mixed) are presented in Appendix B. The steady state cell concentration

and COD results were corrected by taking into account the solids that were dispersed in the liquid and passed through the filter paper during a gravimetric determination. The adjustments are shown in Appendix C. The corrected steady state results are given in Tables 5.5 and 5.6 for all the experiments.

TABLE 5.5

MANUCOL STEADY STATE DATA

DATA NO.	TANK NO.	R_s (days)	R_H (days)	S_o (mg/ℓ COD)	X (mg/ℓ)	COD millipore (mg/ℓ)
1	1	7,76	1,16	1073	1450	181
2	1	9,22	1,16	"	1454	141
3	2	9,00	1,16	"	1737	128
4	2	7,80	1,16	"	1132	196
5	3	9,10	1,12	"	-	139
6	4	4,80	$R_H=R_s$	3320	1140	331
7	5	4,65	"	"	-	287
8	6	4,58	"	"	1079	329
9	9	1,01	"	3000	903	238
10	11	1,73	"	"	841	273
11	12	0,863	"	"	-	-
12	13	2,92	"	"	812	297
13	14	1,46	"	"	872	227
14	15	2,02	"	910	298	103
15	16	3,45	"	"	240	138
16	17	5,84	"	"	233	147

TABLE 5.6

MIXED SUBSTRATES STEADY STATE DATA

DATA NO.	TANK NO.	R_s (days)	R_H (days)	S_o (mg/ℓCOD)	X (mg/ℓ)	CODmillipore (mg/ℓ)
1	23	8,12	1,16	1040	1930	135
2	23	9,34	1,16	"	2100	90
3	24	8,19	1,16	"	2048	84
4	24	8,46	1,16	"	1550	90
5	26	4,74	$R_H=R_s$	3095	1076	249
6	26	4,65	"	"	988	201
7	27	4,65	"	"	761	198
8	28	4,60	"	"	881	187
9	29	1,025	"	3000	1305	160
10	31	2,51	"	"	1320	120
11	32	1,255	"	"	1280	200
12	33	1,86	"	"	1300	185
13	34	0,831	"	"	1235	162
14	35	2,05	"	940	356	77
15	36	3,65	"	"	368	62
16	37	5,13	"	"	381	64

5.5 OBSERVATIONS AND SOURCES OF ERROR

The cell concentration in the effluent (Xe) from tanks operated with settling sections varied for no apparent reason and prevented the attainment of steady cell and substrate concentrations. If a technique could be found for controlling Xe, operation of tanks with settling sections would be a satisfactory method of obtaining kinetic data. As Xe consisted of a dispersed population (see Section 5.6) centrifugation of the effluent may be required. In the experiments loss of cells in the effluent was minimised by collecting the effluent over a 24 hour period and returning the sludge that settled to the units. (Xe recorded in Figures 5.5 and 5.6 are cells that did not settle and were discarded.) Marais [43] recommends this procedure, but there may be objections on the grounds of the sludge losing viability. Ford and Eckenfelder [45], however, reporting their own work and that of Wuhrmann [49] and Westgarth [50]. found that defined periods of anaerobiosis had little effect on the purification capacity of the sludge.

Flow-through tanks were shown to give reproducible steady states, but prolonged periods of operation were required before estimates of steady state values could be made. It was found that at a constant sludge age the COD variation was not excessive, but there was considerable variation in the biological solids concentration. Similar observations were recorded by Gaudy et al. [21] and Chiu et al. [17], as discussed in Section 2.2. These workers attribute this variation to the heterogeneity of the population.

Microscopic examinations (see Section 5.6) showed that the microbial population changed during the experiments, and for the mixed tanks the population was often filamentous, resulting in a bulking sludge. This is known to be caused by excessive growth of filamentous microorganisms in a mixed culture [51], but the cure is not known [45].

Two procedures were attempted to overcome the bulking problem. Firstly, the feed to the tank was stopped for a few days and the tank was aerated vigorously, but this was not very successful. The second method was to stop the feed and

oxygen supply to the tank. Anaerobic conditions had a definite inhibitory effect on the filamentous organisms present, but the settling characteristics of the sludge did not improve significantly. Ford and Eckenfelder [45] recorded similar observations.

The filamentous microorganisms had the effect of binding the other organisms together, and when the organisms were centrifuged a very clear effluent was obtained which generally had a lower COD concentration that that from a non-filamentous tank. Treating a waste water with a filamentous population could thus have advantages.

The apparatus used in this investigation was found to be satisfactory. The effect of intermittent feeding and effluent withdrawal was not evaluated, but Marais [43] found that intermittent operation has little effect on steady state values. Sludge growth on the walls of the tanks, which Ghosh [14] found resulted in erratic substrate and cell concentration fluctuations, was not a problem in this investigation. The tank walls were scraped regularly to ensure that there was no buildup of solids. Difficulty was encountered in keeping the tank contents homogeneous. At one time magnetic stirrers were put into the tanks, but these were not very satisfactory and were discarded. A high air flow rate was used to ensure that there was adequate mixing.

Spot checks showed that there was always an excess of nutrients available (Section 4.5). The only nutrient for which there was a significant difference between feed and effluent concentrations was Mn^{++}. However, the concentration of this ion in the effluent was always high enough for it not to limit growth.

There were insufficient BOD determinations to evaluate growth characteristics in terms of this parameter. Spot BOD checks usually gave results of <10 mg/ℓ, confirming the evidence (see later) that most of the effluent COD was non-degradable.

As far as dual substrates are concerned, the results indicate that, at the sludge ages investigated, both substrates were metabolised simultaneously.

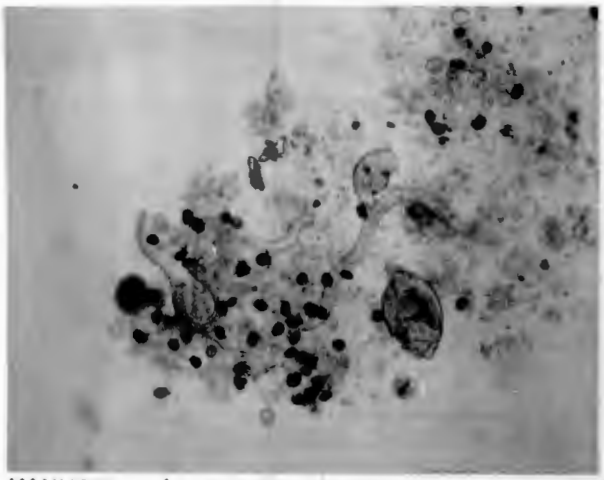
The mixed substrate tanks always had higher sludge concentrations and lower effluent CODs that manucol tanks operated at the same feed strengths. In addition the effluent from the mixed tanks was less turbid than that from the manucol or sugar tanks. It is possible that these observations may be explained by the report of Cooper and Catchpole [52], who note that the breakdown process of a complex waste can be accelerated by the addition of small amounts of glucose to the influent. They note that addition of glucose can improve effluent quality, decrease effluent COD and remove all turbidity from the effluent.

It is further noted that the effect of sugar in the mixed substrate feed was to favour the formation of a filamentous sludge, as shown in Section 5.6. Tischler and Eckenfelder [53] found that a filamentous sludge was capable of a greater rate of assimilation than a flocculated, non-filamentous sludge. Furthermore, in this investigation it was found that a filamentous sludge produced improved effluent quality. If an economical solids-liquid separation for a filamentous sludge could be found, it would be advantageous to use such a sludge in waste water treatment.

5.6 MICROSCOPIC OBSERVATIONS

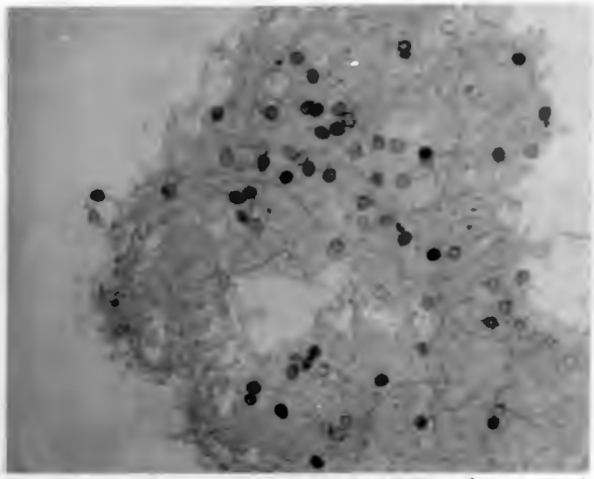
Sludge was examined periodically under a microscope, but no attempt was made to identify the microorganisms. Some information about dual substrate kinetics was deduced from these examinations; this is discussed below.

Representative photographs of the sludge at various stages during this investigation are shown in Figure 5.27. Figure 5.27 (i) is a sludge typical of the tanks operated with settling sections, Figures 5.27 (ii) and (iii) the tanks operated as flow-through units. The manucol tanks usually contained rotifers, stalked and free swimming ciliates, acarinids, worms, flagellates, and bacterial colonies. The dark spots in the photographs were yellow and are believed to be difflugella (testaceae) [54]. These are often found in acid marshy soils. The stages of development of these difflugella are shown in enlarged photographs

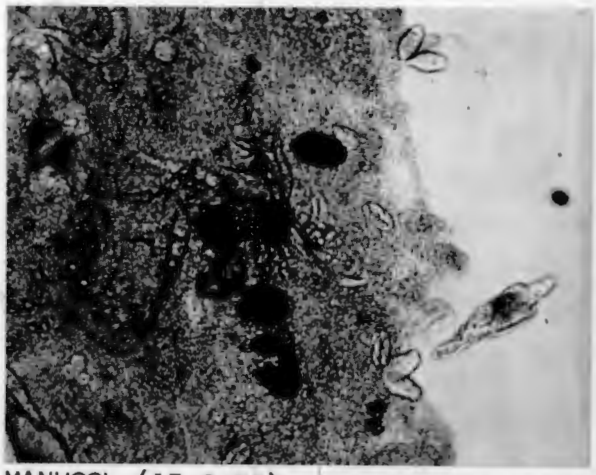


MANUCOL (4.2.74)

FIGURE 5.27 (i)

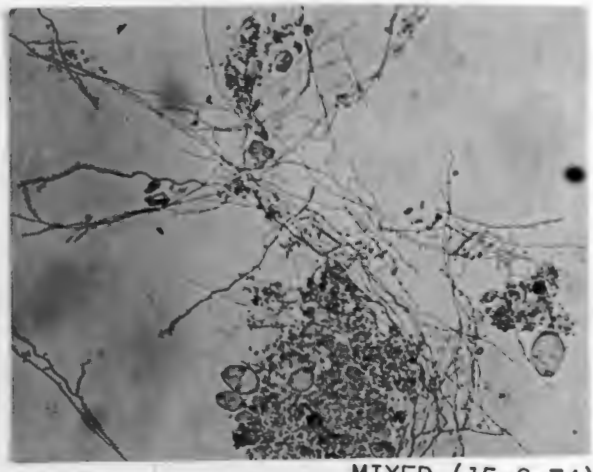


MIXED (4.2.74)

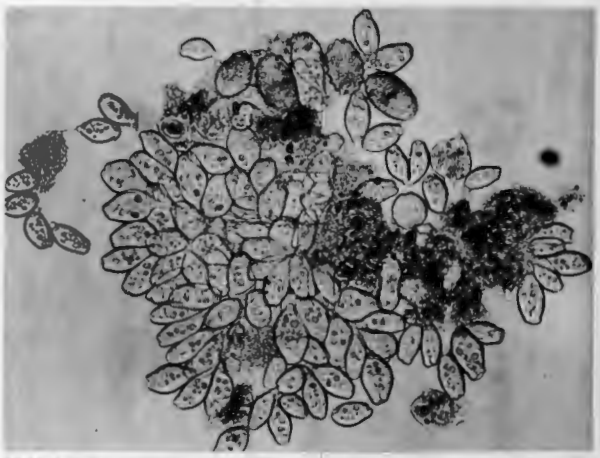


MANUCOL (15.8.74)

FIGURE 5.27 (ii)

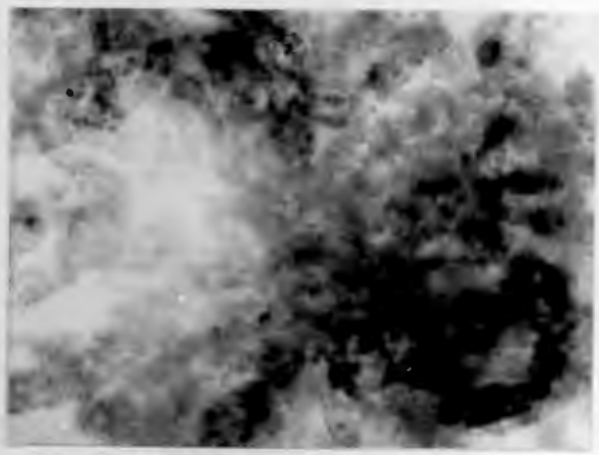


MIXED (15.8.74)



MANUCOL (11.9.74)

FIGURE 5.27 (iii)



SUGAR (29.7.74)

FIGURE 5.27 REPRESENTATIVE MICROPHOTOGRAPHS OF SLUDGE POPULATION (100 X MAGNIFICATION)

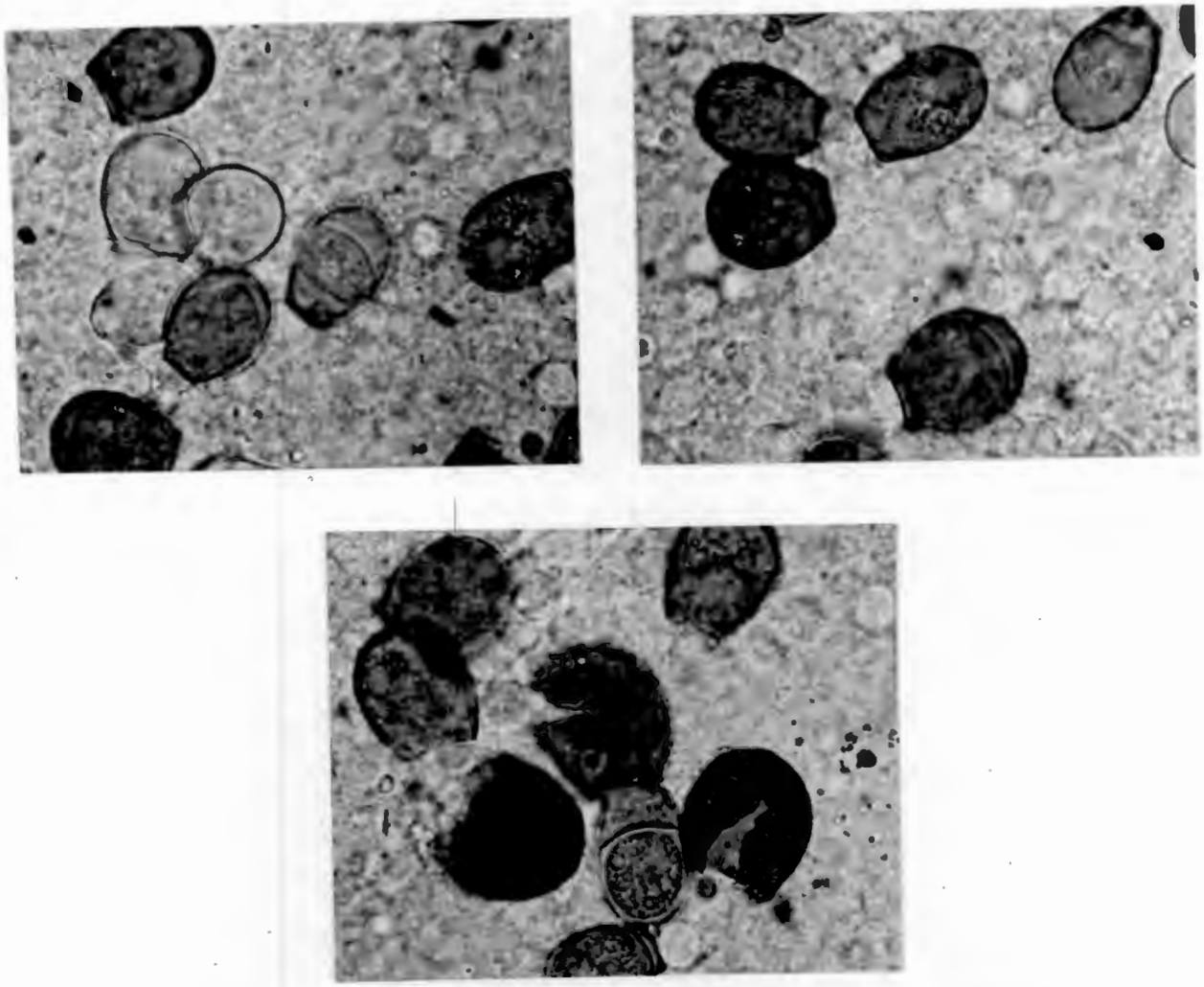


FIGURE 5.28 ENLARGED MICROPHOTOGRAPHS OF "DARK SPOTS"
(1000 X MAGNIFICATION, OIL IMMERSION)

FIGURE 5.29 TYPICAL MICROPHOTOGRAPHS OF EFFLUENTS FROM TANKS
OPERATED WITH SETTLING SECTIONS
(100 X MAGNIFICATION, 4.2.74)

in Figure 5.27. The mixed substrate tanks contained difflugella, rotifers, worms, stalked and free swimming ciliates, bacterial colonies, flagellates and blue-green algae. The sugar tanks contained rotifers, paramecium, healthy bacterial flocs and filamentous zooglea, ciliates and blue-green algae. Difflugella were not found in these tanks.

Difflugella were found when manucol was present, but were not evident in the sugar tanks. The literature survey (Section 2.5.2) suggests that fungal microorganisms do not utilize manucol. It is therefore postulated that these microorganisms metabolised the sugar substrate only.

It is suggested that in the mixed substrate tanks, the following systems were probable:

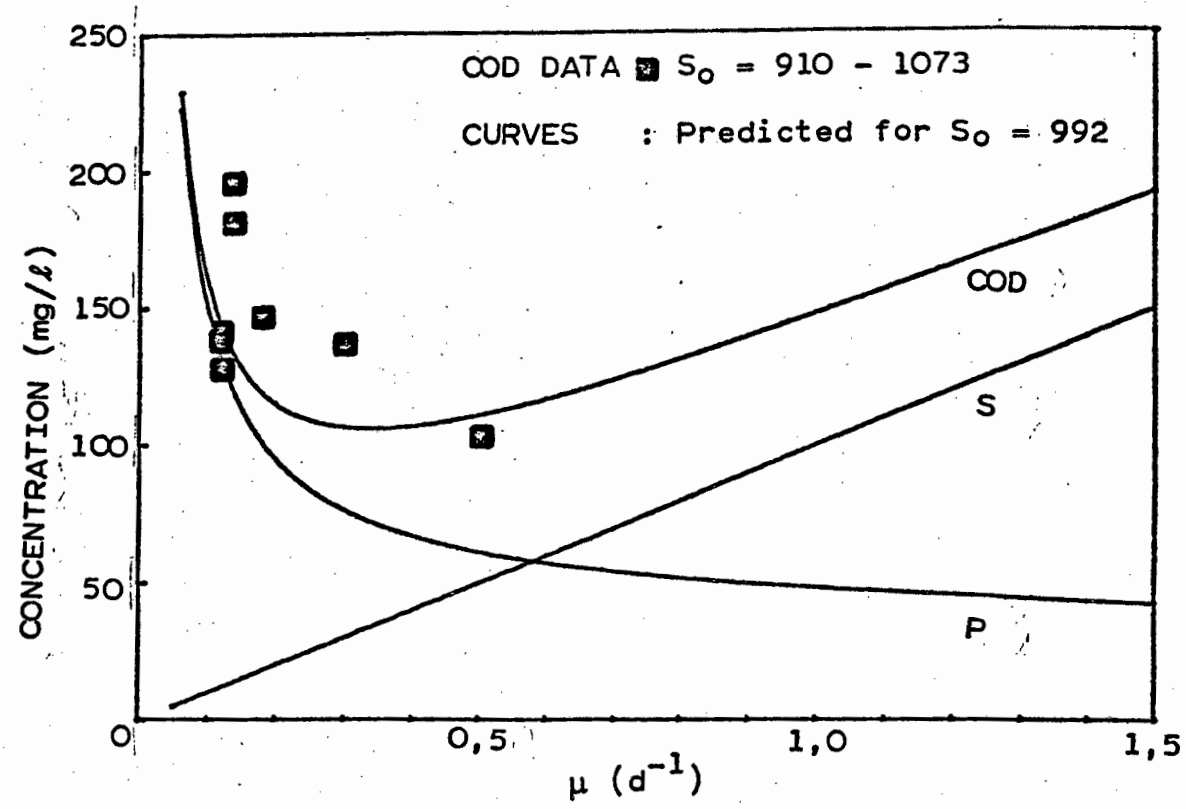
(1) Certain microorganisms were able to metabolise manucol only, and the rest sugar.
(2) Certain microorganisms were able to metabolise manucol only, another fraction of the population could metabolise sugar only, and the remaining fraction was able to utilise both manucol and sugar.

The effluent from the tanks operated with settling sections contained a mass of free swimming microorganisms such as rotifers and flagellates. This is shown in Figure 5.29.

All the tanks had rotifers, which are indicators of an extremely stable biological system [55]. The photographs show that good bacterial flocs were obtained, but poor settling could be attributed to filamentous bacteria or fungi. This was particularly evident in the mixed tanks.

5.7 INTERPRETATION OF RESULTS

The simple theory predicts that a plot of specific growth rate versus substrate concentration should give a hyperbola which passes through the origin (Monod, Equation 1.1). At long sludge ages S should be directly proportional to μ. (Equation 3.21). The COD data in Tables 5.5 and 5.6 were plotted in Figures 5.6 and 5.7 and it was found that they did not follow the predicted S relationship. (The basis on which the curves are drawn is discussed later.) The Monod



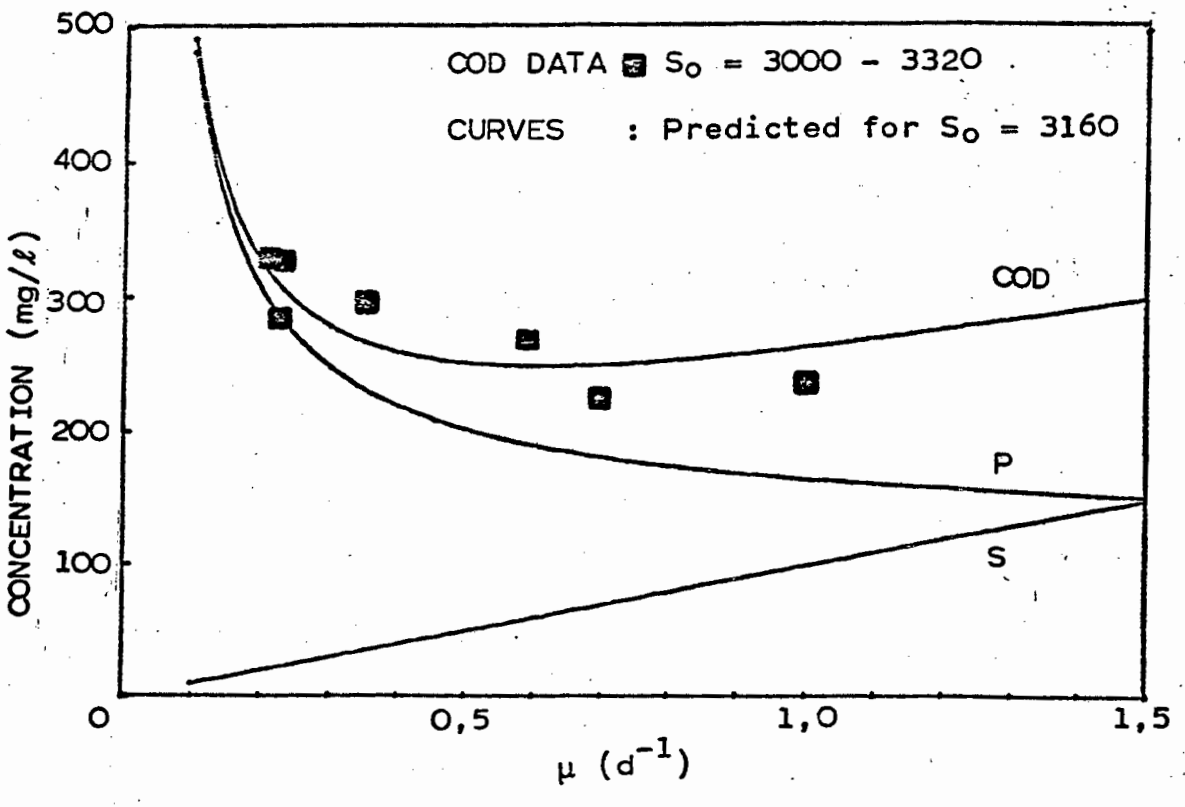


FIGURE 5.6 PREDICTED CONCENTRATION CURVES (S P AND COD) FOR MANUCOL USING THE KINETIC PARAMETERS IN TABLE 5.8 (Effluent COD data for manucol plotted)

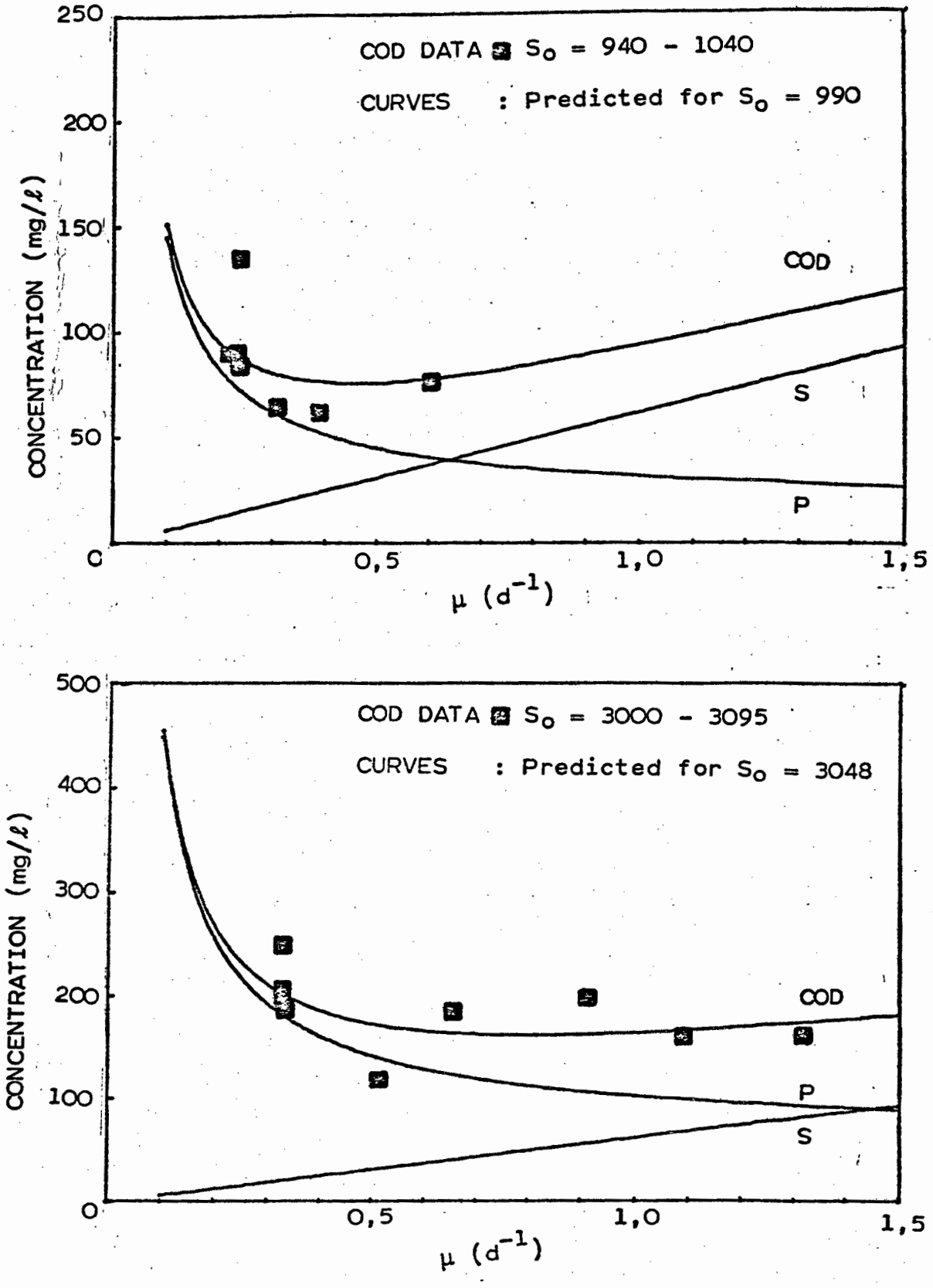


FIGURE 5.7 PREDICTED CONCENTRATION CURVES (S P AND COD) FOR MIXED SUBSTRATES USING THE KINETIC PARAMETERS IN TABLE 5.8 (Effluent COD data for mixed substrates plotted).

model also predicts that effluent substrate concentration is independent of feed strength (see Equation (3.29)). Figure 5.8 showed that effluent COD is a function of feed strength. (It will be shown later that the COD is dependent upon the growth rate as well, and this parameter varies for the data points in Figure 5.8.)

In an attempt to explain these observations, a mathematical model which took into account the concept of product formation was formulated in Chapter 3. It showed (Equation (3.35)) that effluent COD is a function of both specific growth rate and feed concentration, i.e.

$$COD = f\mu + g + \frac{h}{\mu} \qquad (5.3)$$

where $f = \frac{1}{C_1}\ (1 - \alpha Y)$

$g = \alpha Y S_o - \frac{\beta Y}{C_1}$

$h = \beta Y S_o$

(g and h are functions of feed concentration). To establish the parameters Y, b, α, β and C_1, it was assumed initially that endogenous metabolism was small (i.e. b = O) and μ was given by $\mu = \frac{1}{R_s}$ (see Equation (3.26)). αY, βY and C_1 were found by the method of least squares (see Appendix E). The results are given in the Table below.

Table 5.7 : Values of kinetic parameters when b = O

	αY	βY	C_1	correl. coefft, r
Manucol	O,O465	O,O100	O,O102	O,93
Mixed	O,O320	O,OO66	O,O18	O,91

The high correlation coefficients suggested that Equation (5.3) could be used to correlate the data, and the model in Chapter 3 appeared to be satisfactory.

The cell concentration data were then used to estimate the endogenous metabolism constant, b, by manipulation of the equations of the model. Equation (3.27) was rearranged to give

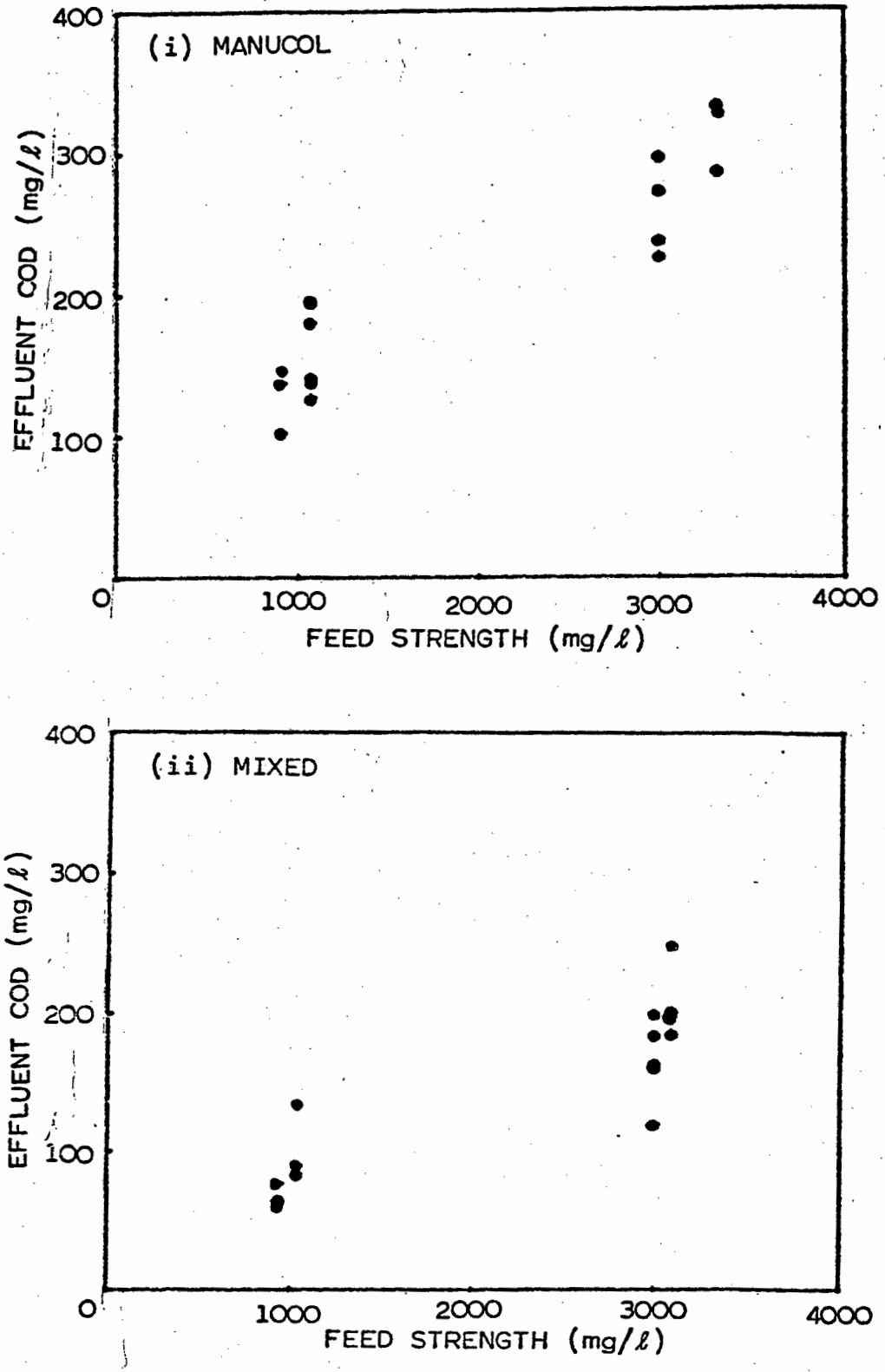


FIGURE 5.8 EFFECT OF FEED STRENGTH ON EFFLUENT COD.

$$\frac{S_o - S}{R_H X} = \frac{1}{Y}\frac{1}{R_S} + \frac{b}{Y} \qquad (5.4)$$

As COD was not a measure of S, the growth limiting substrate, it was necessary to obtain Equation (5.4) in terms of COD. As COD = P + S (Equation (3.30)), it followed that

$$\frac{S_o - COD}{R_H X} = \frac{S_o - S}{R_H X} - \frac{P}{R_H X} \qquad (5.5)$$

Substitution of Equations (5.4), (3.25) and (3.26) into this equation gave

$$\frac{S_o - COD}{R_H X} = \left(\frac{1}{Y} - \alpha\right)\frac{1}{R_S} + \left(b\left(\frac{1}{Y} - \alpha\right) - \beta\right) \qquad (5.6)$$

For most of the data, $R_H = R_S = R$, hence

$$\frac{S_o - COD}{X} = \left(\frac{1}{Y} - \alpha\right) + \left(b\left(\frac{1}{Y} - \alpha\right) - \beta\right) R \qquad (5.7)$$

$\frac{S_o - COD}{X}$ was plotted versus R in Figure 5.9, and the results were:

	$\left(\frac{1}{Y} - \alpha\right)$	$b\left(\frac{1}{Y} - \alpha\right) - \beta$	Correl. Coefft. (r)
Manucol	3,044	0,0	0,0
Mixed	1,9458	0,2018	0,64

The manucol cell concentration data were scattered and could best be represented by an average value. It is unusual to get a good correlelation using Equation (5.7); for instance the data of Grady and Williams [25] gave a correlation coefficient of 0,16 and it may have been preferrable to use an average value other than the values obtained from their poor correlation. Y, b, α and β could then be calculated; e.g. for mixed substrates:

$$\left(\frac{1}{Y} - \alpha\right) = 1,9458 \qquad (5.8)$$

$$b\left(\frac{1}{Y} - \alpha\right) - \beta = 0,2018 \qquad (5,9)$$

$$\therefore \quad 1,9458b - \beta = 0,2018 \qquad (5,10)$$

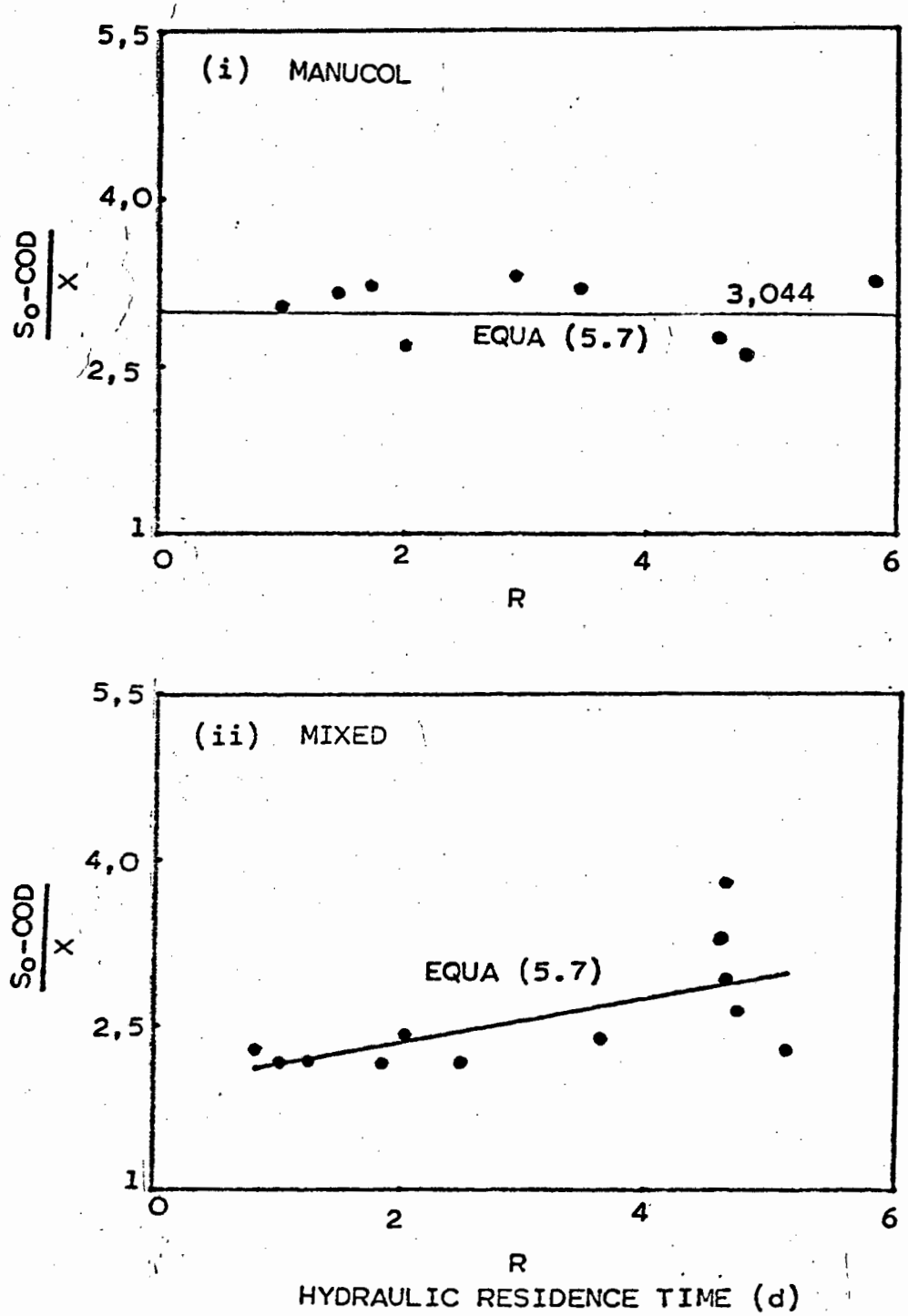


FIGURE 5.9 DETERMINATION OF SLUDGE PARAMETERS.

Also $\alpha Y = 0,0320$ (5.11)

$\beta Y = 0,0066$ (5.12)

Equation (5.8) gave

$1-\alpha Y = 1,9458Y$

$\therefore \quad 1-0,0320 = 1,9458Y$

$\therefore \quad Y = 0,497$

$\therefore \quad \alpha = 0,064$

$\therefore \quad \beta = 0,013$

$\therefore \quad b = 0,11$

The parameters for manucol were Y = 0,313 mg cells/mg COD, $b = 0,01\ d^{-1}$, $\alpha = 0,148$, $\beta = 0,032\ d^{-1}$. The values for b could be substituted into Equation (3.26) and the data fitted to Equation (5.3) with the new value for μ. The calculations illustrated above were repeated to give new values for the parameters Y, b, α and β. The procedure was repeated until there was no further significant change (3 iterations required). The final results are given in Table 5.8.

Table 5.8 : Kinetic parameters for manucol and mixed substrates

	Y mgcells/ mgCOD	b d^{-1}	α mg-product mg-cells^{-1}	β mg-product mg-cells^{-1} d^{-1}	C_1 $\ell mg^{-1} d^{-1}$	Correl. Coefft. r
Manucol	0,314	0,012	0,138	0,035	0,010	0,93
Mixed	0,503	0,117	0,043	0,025	0,016	0,91

These parameters were substituted into the equations for S, P and COD proposed in Chapter 3. The relationships were plotted in Figures 5.7 and 5.8; these showed that the model was able to correlate the COD data satisfactorily.

It is interesting to compare these findings to those of Grady and Williams [25]. They proposed the empirical relationship

$$COD = K^{1} \frac{S_o}{R} + K^{11} S_o \qquad (5.13)$$

to describe the effect of residence time and feed strength on effluent COD. The equation was rearranged (Equation 5.14))

and applied to the data of this investigation. The correlation was poor.

$$\frac{COD}{S_o} = \frac{K^1}{R} + K^{11} \qquad (5.14)$$

The data of Grady and Williams were then tested according to the model used to describe the manucol and mixed substrate systems. Their data could be fitted to the equations of the model with a correlation coefficient of 0,86. However, the value of determined from the analysis was negative, and this resulted in a negative COD being predicted for low μ values. It is possible that the increase of COD at low μ values (high R_s) observed for manucol and mixed substrates was not observed during their experiments because they were conducted at high μ values (longest R_s was 0,5 days) (See Figure 5.10). Thus, the data of this thesis did not fit their equation, while the application of the equations of this study to their data was inconclusive.

5.8 DISCUSSION

This investigation set out to examine the kinetics of growth of activated sludge on a dual substrate feed. It was found during substrate metabolism that the number of substrates in the process increased due to product formation. This made measurement of growth on the two original substrates complicated because COD was not only composed of the growth limiting substrate concentration, but excretion products as well. The model proposed in Chapter 3 to describe COD concentration in the activated sludge process was able to correlate the experimental data satisfactorily. The kinetic parameters obtained from the correlations were used to calculate substrate and product concentrations, and the results, plotted in Figures 5.6 and 5.7, showed that effluent COD consists primarily of microbial excretion products.

The implications of these findings on waste water treatment processes are significant. It is commonly believed that the longer the sludge age, the lower is the effluent COD. Figure 3.2 suggests that there is an optimum sludge age at which the lowest effluent COD is obtained. This optimum can

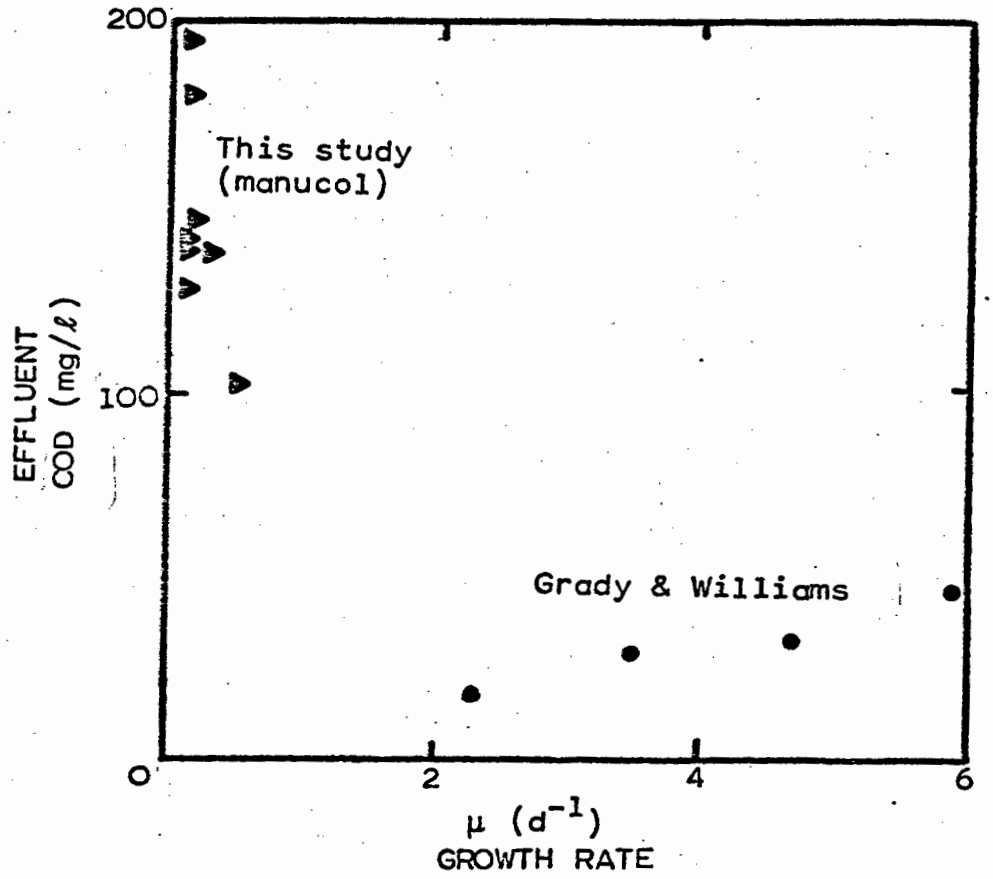


FIGURE 5.10 SPECIFIC GROWTH RATES INVESTIGATED IN THIS STUDY AND BY GRADY AND WILLIAMS
(Data for 1000 mg/ℓ COD feed strength)

be obtained by differentiating Equation (5.3) with respect to μ, and setting equal to zero, i.e.

$$\frac{d\ COD}{d\mu} = 0 \qquad (5.14)$$

$$\therefore \quad \mu_{opt} = \sqrt{\frac{h}{f} S_o}$$

$$= \sqrt{\frac{C_1 \beta\ Y S_o}{1-\alpha Y}}$$

The values for manucol and mixed substrates are given in Table 5.9.

Table 5.9 : Optimum operation of an activated sludge process treating manucol and mixed substrates

	S_o mgCOD/ℓ	μ_{opt} d^{-1}	S_o mgCOD/ℓ	μ_{opt} d^{-1}
Manucol	992	0,338	990	0,451
Mixed	3160	0,603	3048	0,792

The concept of product formation was able to explain several observations made during the course of this investigation. Some of these were:

(a) During acclimatisation experiments, the COD was never zero, even when the population was acclimatised to the substrate it was being fed.

(b) Residual COD values were measured during batch experiments for most of the substrates examined.

(c) Aeration of MLSS taken from some of the tanks did not result in a COD change for several days, suggesting that the COD was not composed of readily degradable carbohydrates.

Product formation offers an explanation for the residual COD measured by other workers, (see Table 2.2). It also explains the observations of Chudoba [29] - reported in Section 2.3 - that, at a sludge age of 50 days, effluent COD was double that obtained at a sludge age of 10 days.

Any model of a biological process is necessarily a simplified description of the complex reactions and interactions that occur. A great deal of attention has been focussed on computer simulation of some of these interactions, e.g. Curds [56]. These have shown the complexity and variety of interactions that are likely to occur in any biological system. Numerical values for kinetic constants are necessarily imprecise because, even under steady state conditions, the system is an internally dynamic one and there is a continuously shifting predominance of species [57]. For example, Gaudy and his associates [58] collected data over 8 years and concluded that, even under the most carefully controlled experimental conditions, the yield value for a heterogeneous population cannot be expected to be constant.

The parameters determined in this study can also not be regarded as absolute constants. They were measured for a manucol-sugar waste only, and their use is necessarily restricted.

CHAPTER 6

CONCLUSIONS AND RECOMMENDATIONS

6.1 CONCLUSIONS

In most biological treatment processes the waste stays in contact with the microorganisms for a sufficiently long time for readily degradable carbohydrates to be utilised. The engineer is concerned with reducing the effluent COD from such processes to a low value. Most design equations at present are based on degradable substrate concentration. However, the results of this investigation quantitatively show that it is not degradable substrate that is responsible for effluent COD, but product formed as a result of biological activity. This evidence indicates that the purification efficiency of a waste treatment process can be seriously affected by product formation, which can convert a waste with only a few carbon sources to a heterogeneous mixture of compounds which are very much less easily degraded than the original compounds. Hence, a quantitative description of product formation during a biological treatment process is essential for optimisation and design purposes.

A model for product formation proposed in this study was able to explain several observations from this and other investigations which could not be explained by the degradable substrate theory.

The conclusions that can be drawn from this work are:

1. Feed concentration has a significant effect upon effluent COD from an activated sludge reactor. This is true for
   - a reactor operated with recycle of microorganisms
   - a reactor operated as a flow-through unit
   - a multicomponent feed
   - a single substrate feed.
2. The effect of feed concentration may be satisfactorily modelled for the systems investigated by incorporating the

Luedeking and Piret hypothesis for product formation into existing activated sludge theory.

3. When COD is used as a measure of the growth limiting substrate concentration (S), the Monod equation is not applicable, particularly at long sludge ages.

4. There is an optimum sludge age for each feed concentration that will achieve minimum effluent COD.

5. Sugar and manucol are consumed simultaneously at the sludge ages used in this investigation.

6.2 RECOMMENDATIONS

The data obtained in this study were in good agreement with the mathematical model proposed, but do not alone furnish sufficient evidence for acceptance of the model. It is recommended that other substrates be examined over a wide range of sludge ages and feed concentrations to confirm the validity of the product theory or expose its shortcomings.

The technique proposed in this study for kinetic parameter evaluation enables an estimate to be made of the amount of product and undegraded substrate present at any sludge age. In future experiments individual substrate concentrations should be measured to confirm the validity of these kinetic parameters.

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APPENDIX A

DATA FROM BATCH CULTURE EXPERIMENTS

TABLE A.1

MANUCOL BATCH RESULTS

Initiation Date: 26.8.1973

Temperature: 18,5± 0,2°C

pH: 6,98 at start of batch experiment; decreased to 6,82 at end of experiment.

DO: remained practically constant at 9,5 ppm for the duration of the experiment.

BATCH TIME (h)	COD (mg/ℓ)	X (mg/ℓ)
0,0	981	94,5
2,5	908	114
13,25	901	113
15,17	858	122
16,75	872	114
18,17	861	116
20,17	856	127
22,17	847	

TABLE A.1 (Contd)

BATCH TIME (h)	COD (mg/ℓ)	X (mg/ℓ)
24,67	825	110
26,92	827	126
28,58	819	116
32,92	801	109
37,17	794	130
40,17	789	
42,92	799	129
48,17	731	164
51,42	718	175
61,17	605	193
62,67	585	197
66,0	536	211
67,17	510	225
69,42	447	238
72,50	383	281
74,83	348	271
76,83	312	260
78,58	266	302
81,66	200	329
87,17	163	328

TABLE A.2

SUGAR BATCH RESULTS

Initiation Date: 19.8.1973

Temperature: 18,1± 0,2°C

pH: 7,01 at start of batch experiment; decreased to 6,82 at end of experiment.

DO: Refer to Figure A.1. DO was constant at about 9 ppm until 13,88 hours, then dropped to about 7 ppm until 21,42 hours, when the DO rose sharply. Figure A.1 confirmed the phenomenon of 3 phase growth reported in the text. Phase 1, the lag phase, lasted for 13,88 hours and phase 2, the log growth phase, lasted for 7,54 hours. The duration of the phases was comparable to that determined by cell concentration measurements. (see text.)

BATCH TIME (h)	COD (mg/ℓ)	X (mg/ℓ)
0,00	1027	19,6
8,58	970	54
9,33	904	54
10,25	988	63
11,17	927	
12,08	939	79
13,92	936	93
15,58	704	131
16,58		166
17,87	531	204
19,42	408	289
20,37	305	353
21,67	184	352
22,42	294	362
23,25	92	381
23,58	108	
23,90		380

TABLE A.3

PECTIN BATCH RESULTS

Initiation Date: 13.8.1973

Temperature: 18,0± 0,1°C

pH: Refer to Figure A.2. 6,98 at start of experiment; decreased to 6,60 at end of experiment.

DO: Always greater than 5 ppm

BATCH TIME (h)	COD (mg/ℓ)	X (mg/ℓ)
0,00	950	22,4
8,83	793	
9,87		125
11,42	685	
12,33	612	
14,28	457	247
15,00	350	
16,20	321	335
16,83	270	
17,75	143	408
18,42	146	438
20,20	95	432
22,15	91	435

TABLE A.4

STARCH BATCH RESULTS

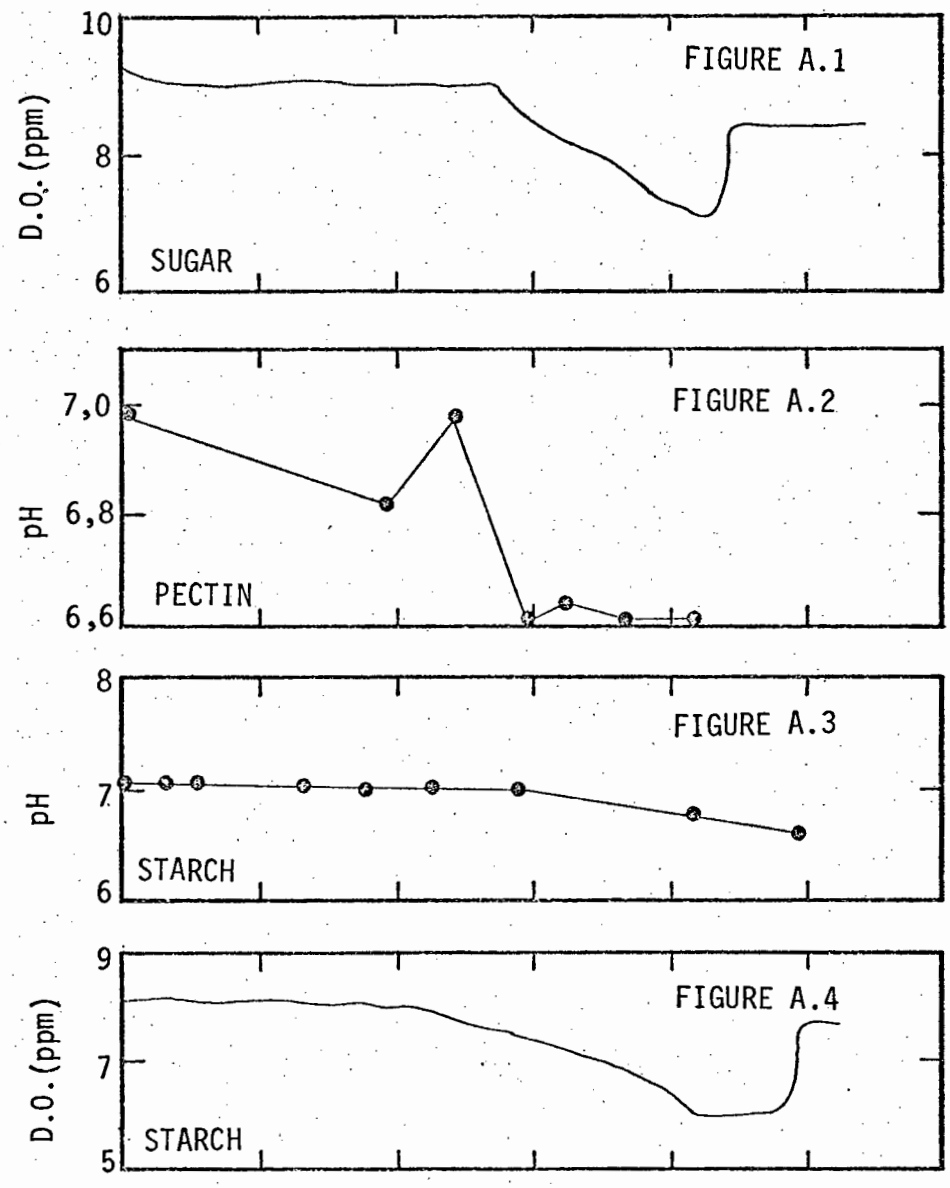
Initiation Date: 30.8.1973

Temperature: 19,3± 0,7°C

pH: Refer to Figure A.3. 7,08 at start of experiment; decreased to 6,59 at end of experiment.

DO:. Refer to Figure A.4. DO dropped steadily until 24,2 hours, and then rose sharply when all the substate was exhausted. The cell concentration data indicated that the log growth phase was complete after 23 hours batch time. (see text).

BATCH TIME (h)	COD (mg/ℓ)	X (mg/ℓ)
0,00	750	16,00
1,03	744	
2,07	738	
3,12	722	
4,28	724	
5,62		
7,12	740	
8,02	752	
9,12	743	
10,37	740	
11,00	669	
11,7	664	
12,62	637	
13,97	581	
14,88	551	
15,92	511	
17,25	464	
18,28	452	
19,20	397	186
20.62	323	
22,68	195	
24,20	87	387
25,17	0	355



FIGURES A.1–A.4 BATCH EXPERIMENTAL RESULTS

APPENDIX B

EXPERIMENTAL DATA FROM CONTINUOUS CULTURE RUNS

B.1 DETAILS OF EXPERIMENTS WITH MANUCOL AS SUBSTRATE

TANK NO: 1 TANK VOLUME: 5,810 ℓ

SUBSTRATE: Manucol

TANK DESCRIPTION: Settling compartment for effluent included.

HISTORY OF MICROORGANISMS: Feed taken from batch acclimatised flask.

OPERATING CONDITIONS: Hydraulic age of 1,16 days set by the automatic feeding system. Feed four times an hour, 10 min being allowed for the feeders to fill, and 5 min to discharge. The feed concentration was 1073 mg/ℓ COD.

Sludge age set by withdrawing 0,3ℓ of mixed liquor from tank every 12 hours. The baffle was lifted to allow the entire tank contents to be as homogeneos as possible before sludge withdrawal.

The effluent was collected in a bucket and allowed to stand for a day. The clear liquid in the bucket was poured off and analysed for solids concentration. The solids which had settled in the bucket were returned to the tank. The solids concentration measured in the effluent, X_e, was a measure of the non-settleable microorganisms, and was used to calculate the sludge age.

SAMPLE CALCULATION: X_e is recorded in the following tables on the day to which it applies. For example, in Table B.1 X_e is given as 13 mg/ℓ for day 70, but this was measured on day 71. Generally X_e was measured every second day. Thus, in Table B.1, X_e for day 65 (i.e. 79 mg/ℓ) was the average X_e for days 64 and 65, and R_s was calculated for both these days using X_e = 79 mg/ℓ.

The volume of MLSS withdrawn was recorded every day, and was usually 0,6 ℓ/d. A sample calculation of R_s for day 70 of Table B.1 is illustrated below.

$$R_s = \frac{VX}{WX + EX_e} \qquad (3.18)$$

V = 5,810 ℓ
W = 0,60 ℓ/d
E = 5,00 ℓ/d as R_H = 1,16 d
X (for day 70) = 1153 mg/ℓ
X_e (for day 70) = 13 mg/ℓ

Therefore R_s = 8,85 d

EXPERIMENTAL DATA: Tabulated in Table B.1

TABLE B.1

EXPERIMENTAL RESULTS FOR TANK NO.1 OPERATING ON MANUCOL AS SUBSTRATE

TIME (days)	COD (mg/ℓ)	X (mg/ℓ)	pH	SVI	X_e (mg/ℓ)	R_s (d)
0-start						
1	561	736	7,58			8-nominal
4			7,12			
6	178	784				8
12	190	2030				8
13	228	2041	6,62			8
14	296	1910				8
16	328	1436	6,18	139	233	8
19	134	1545				8
25			6,0			
26	115	1533		104		6,26
27	159	1506	6,25	100		6,22
30		1243		88	93	5,82
31	227	1110	6,28	77		7,97
32			6,01		23	
33	241	1477	6,35	68	54	31,78
34	211	1523				30,00
36	249	1435		59		7,21
37		1326			59	7,06
38	405	1030	5,60	68	18	8,45
39	235	1288				7,50
40	159	1460			45	7,70
41	182	1377			35	7,99
42	154	1368				7,90
43	151	1385	5,90			7,92
44	157	1367				13,35
45	173	1483				8,02
46					37	
47	232	1323				7,85
53					82	
54	249	1431				7,30
56	236	1449			56	7,32
57					27	
58	286	1500	5,00			8,42
59	587	1414				7,99
60					36	
61	673	1198			23	8,35
62	456	1244				7,76
63	159	1383	5,43	56	37	7,92
64	155	1289				6,41
65					79	
66	173	1249	5,03	40		7,33
67					48	
68					50	
69	197	1115	5,28	39		8,83
70	131	1153	5,42		13	8,85
71	122	1298			40	7,70
72	127	1317	5,43	38		9,27
73	120	1388				9,29
74					7	
75	119	1483	5,82			9,42
76	127	1604			5	9,44
77	166	1535				8,69
78	142	1404			21	8,61
79	142	1522		39		8,68
80			6,60			
81	194	1385			3	9,51
82	226	1364		51		9,51
83	212	1367	6,65			9,51
84	211	1299				9,50

Additional Information for Tank 1:

S_o = 1073 mg/ℓ (Range: 1038 - 1139)
% volatility = 91,65% (90,68 - 92,73)
[O] = 4,5ppm (2,8 - 6,5)
tank temp = 20,36°C (20,1 - 20,7)

ANALYSIS: See text. Steady state estimates:

Period considered (days)	R_s (d)	COD (mg/ℓ)	X (mg/ℓ)	SVI
31 - 58	7,76	209	1413	60
72 - 84	9,22	162	1424	40

TANK NO: 2 TANK VOLUME: 5,800ℓ

SUBSTRATE: Manucol

TANK DESCRIPTION: Settling compartment for effluent included.

HISTORY OF MICROORGANISMS: Seed taken from batch acclimatised flask.

PERIOD OF OPERATION: 10.11.73 - 2.2.74

OPERATING CONDITIONS: As for Tank No: 1. The hydraulic age was 1,16 days.

EXPERIMENTAL DATA: Tabulated in TABLE B.2

NOTES: As for TANK NO: 1.

TABLE B.2

EXPERIMENTAL RESULTS FOR TANK NO: 2 OPERATING ON MANUCOL AS SUBSTRATE

TIME (days)	COD (mg/ℓ)	X (mg/ℓ)	pH	SVI	X_e (mg/ℓ)	R_s (d)
0-start						
1	107	497	7,68			8-nominal
4			7,48			
6	227	1396				8
12	227	744				8
13	600	670	6,80			8
14	600	534				8
16	600	304	6,48	82	205	8
19	403	445				8
25			6,76			
26	143	1462		123		6,43
27	141	1591	6,45	114		6,59
30		1336		120	81	6,25
31	359	1229	6,60	99		8,56
32			6,32		13	
33	302	1631	6,60	87	26	72,77
34	212	1820				"
36	132	1782		92		9,67
37		1781			0	9,67
38	185	1547	5,80	96	0	9,67
39	156	1659				9,67
40	148	1672			0	9,67
41	155	1666			19	8,83
42	124	1655				8,08
43	134	1598	5,50			8,03
44	131	1662				13,90
45	132	1694				8,11
46					39	
47	89	1697				8,11
53					5	
54	173	1664				9,62
56	216	1845			1	9,62
57					31	
58	333	1947	5,10			8,19
59	711	1716				8,03
60					42	
61	873	1241			54	7,09
62	345	1368				8,22
63		1487	6,69	67	29	8,32
64	177	1431				8,70
65					19	
66	189	1388	6,40	36		7,50
67					48	
68					74	
69	252	1215	5,93	33		6,21
70	244	1165	5,82		81	6,12
71	253	1027			75	6,01
72	280	971	6,11	41		6,89
73	331	1103				7,13
74					47	
75	242	916	6,44			8,37
76	219	1131			17	8,59
77	210	1106				8,63
78	221	1212			16	8,71
79	240	1164				8,25
80			5,74	72		
81	217	1234			24	8,32
82	188	1134		123		8,22
83	140	1113	6,45			8,19
84	151	936				7,96

ADDITIONAL INFORMATION FOR TANK 2:

S_o = 1073 mg/ℓCOD (1038 - 1139)
% volatility = 91,61% (90,13 - 92,89)
[O] = 5,5 ppm (3,6 - 7,8)
tank temp = 20,2°C (20,0 - 20,4)

ANALYSIS: See text. Steady state estimates:

Period Considered (days)	R_s (d)	COD (mg/ℓ)	X (mg/ℓ)	SVI
33 - 58	9,00	148	1708	94
70 - 84	7,80	226	1093	62

TANK NO: 3 TANK VOLUME: 5,605ℓ

SUBSTRATE: Manucol

TANK DESCRIPTION: Settling compartment for effluent included.

HISTORY OF MICROORGANISMS: Seed taken from batch acclimatised flask.

PERIOD OF OPERATION: 10.11.73 - 2.2.74

OPERATING CONDITIONS: As for Tank No. 1. The hydraulic age was.1.12 days.

EXPERIMENTAL DATA: Tabulated in Table B.3

NOTES: As for tank No. 1

TABLE B.3

EXPERIMENTAL RESULTS FOR TANK NO: 3 OPERATING ON MANUCOL AS SUBSTRATE

TIME (days)	COD (mg/ℓ)	X (mg/ℓ)	pH	SVI	X_e (mg/ℓ)	R_s (d)
0-start						
1	172	457	7,69			8-nominal
4			7,48			
6	170	923				8
12	385	1919				8
13	510	1996	6,48			8
14	600	1870				8
16	600	1669	6,42	128	71	8
19	138	1993				8
25			6,30			
26	141	2100		136		7,58
27	137	2094	6,40	128		7,58
30		1889		100	48	7,45
31	170	1724	6,67	104		7,97
32			6,32		27	
33	314	2052	6,75	77	66	6,91
34	261	1771				16,50
36	145	1805		94		9,05
37		1790			7	9,05
38	164	1596	6,68	93	2	9,25
39		1584				9,29
40	148	1505			1	9,29
41		1485			6	9,19
42		1537				9,00
43	162	1351	6,20			8,96
44	161	1396				17,24
45	165	1471				8,99
46					7	
47	162	1448				8,98
53					113	
54	158	2428				8,94
56	140	1638			13	8,76
57					0	
58	175	1875	6,40			8,18
59	183	2054				8,27
60					32	
61	201				178	
62	172					
63	159	1805	6,26		166	5,29
64	179	1646				6,33
65					94	
66	279	1357	5,39	35		7,02
67					54	
68					59	
69	228	1270	6,01	37		7,40
70	276	1311	5,95		40	7,45
71	186	1293			7	8,94
72	210	1435	6,15	42		9,03
73	234	1433				9,03
74					6	
75	205	1356	6,60			8,18
76	204				23	
77	237	1254				6,43
78	202	1215			68	6,37
79	184	1164		210		6,28
80			6,00			
81	178	900			33	7,16
82	171	807		136		6,97
83	113	980	6,11			7,29
84	131	893				7,14

Additional Information for Tank 3:

S_o = 1073 mg/ℓ (1038 - 1139)

% volatility = 91,69% (91,1 - 92,11)

DO = 4,7ppm oxygen (2,2 - 6,7)

tank temp = 20,3°C (19,9 - 20,6)

ANALYSIS: See text. Steady state extimates:

Period Considered (days)	R_s (d)	COD (mg/ℓ)	X (mg/ℓ)	SVI
36 - 59	9,10	160	-	93

TANK NO: 4 TANK VOLUME: 5,810ℓ

SUBSTRATE: Manucol

TANK DESCRIPTION: Flow-through system

HISTORY OF MICROORGANISMS: Previously operating as Tank no. 1.

PERIOD OF OPERATION: 12.2.74 - 9.6.74

OPERATING CONDITIONS: The hydraulic residence time (equal to sludge age) was set by the automatic feeding system. Feed once an hour, 30 mins being allowed for the feeders to fill, and 30 mins to discharge. The hydraulic age was 4,80 days until day 80, then 4,84 days.
The feed concentration was 3320 mg/ℓ COD.

EXPERIMENTAL DATA: Tabulated in Table 4.

TABLE B.4

EXPERIMENTAL DATA FOR TANK NO: 4 OPERATING ON MANUCOL AS SUBSTRATE

TIME (days)	COD-g.f. (mg/ℓ)	COD-milli (mg/ℓ)	X (mg/ℓ)	pH
0-start				
2	984		714	5,63
3				6,05
4	254		1208	
6	260		1204	5,45
7	273		1244	
8	306		1155	5,35
9	372		1031	5,24
10	368	307	1055	
13			1059	
14	382		985	
22				5,51
23	451		1262	
24	353		1252	6,20
27	353		1094	6,05
28	398		848	
41			1195	
47	269		1057	6,72
49				6,82
51	240		1151	7,18
52				7,30
55	571		1294	7,31
76	420		1468	7,20
82	634		1203	
84	585		1139	
86	370	165	936	6,60
89	375		1162	
91	375	375	846	6,40
93	279	270	876	
94	450	360	875	
97	321	267	1036	7,32
100	486		844	
102	1100		582	
109	6100		499	6,22
115	350		654	
117			1245	

Additional Information for Tank 4:

S_o = 3320 mg/ℓ COD
% volatility = 88,74% (86,97 - 92,39)
[O] = 5,9 ppm oxygen (2,4 - 7,4)
tank temp = 19,43°C (19,0 - 19,9)
$\frac{COD_{mi}}{COD_{gf}}$ = 0,813

ANALYSIS: For the first 100 days of operation, the average cell concentration was 1081 mg/ℓ, (Range: 844 - 1262). The average COD was 381 mg/ℓ, but there was considerable variation, between 254 and 585 mg/ℓ.

TANK NO: 5 TANK VOLUME: 5,800ℓ

SUBSTRATE: Manucol

TANK DESCRIPTION: Flow through system

HISTORY OF MICRO-ORGANISMS: Previously operating as Tank No. 2

PERIOD OF OPERATION: 12.2.74 - 29.4.74

OPERATING CONDITIONS: As for Tank No 4. The hydraulic age (equal to sludge age) was 4,65 days.

EXPERIMENTAL DATA: Tabulated in Table 5.

TABLE B.5

EXPERIMENTAL RESULTS FOR TANK NO: 5

OPERATING ON MANUCOL AS SUBSTRATE

TIME (days)	COD-g.f. (mg/ℓ)	COD-milli (mg/ℓ)	X (mg/ℓ)	pH
O-start				
2	984		213	6,0
3				6,31
4	985		599	
6	504		682	7,08
7	332		722	
8	293		869	6,98
9			939	6,90
10	288	277	1104	
13			1265	
14	362		1257	
22				6,14
23	357		1571	
24	371		1669	6,32
27	337		1759	6,28
28			1674	
41			1433	
47	279		1232	5,20
49				5,38
51	280		1009	5,80
52				6,12
55	408		1180	7,30
76	520		675	7,45

Additional Information for Tank 5:

S_o = 3320 mg/ℓ COD

% volatility = 88,14% (82,30 - 93,0)

[O] = 5,8 ppm oxygen (1.9 - 8,4)

tank temp = 19,39°C (18,8 - 13,9)

ANALYSIS: COD results steady for days 7 - 55 at 331 mg/ℓ (280 - 371) Although the COD was constant, the cell concentration changed considerably: in 27 days it built up from 213 to 1759 mg/ℓ, and then, during the next 30 days, it slowly decreased to 675 mg/ℓ. No extimate of an averate is possible.

TANK NO: 6 TANK VOLUME: 5,605ℓ

SUBSTRATE: Manucol

TANK DESCRIPTION: Flow through system

HISTORY OF MICRO-ORGANISMS: Previously operating as Tank No 3.

PERIOD OF OPERATION: 12.2.74 - 29.4.74

OPERATING CONDITIONS: As for Tank No 4. The hydraulic age (equal to sludge age) was 4,58 days.

EXPERIMENTAL DATA: Tabulated in Table 6.

TABLE B.6

EXPERIMENTAL RESULTS FOR TANK NO: 6

OPERATING ON MANUCOL AS SUBSTRATE

TIME (days)	COD-g.f. (mg/ℓ)	COD-milli (mg/ℓ)	X (mg/ℓ)	pH
O-start				
2	700		506	6,21
3				6,46
4	331		740	
6	365		877	6,90
7	343		835	
8	349		851	6,62
9	371		754	6,42
10	406	388	744	
13			817	
14	495		993	
22				6,10
23	443		1054	
24	384		1015	6,40
27	344		1039	6,60
28	342		984	
41			1042	
47	984		445	5,50
49				5,72
51	>1200		310	6,20
52				7,0
55	1206		525	6,75
76	361		1556	7,70

Additional Information for Tank 6:

S_o = 3320 mg/ℓ COD

% volatility = 88,17% (85,2 - 94,26)

[O] = 7,0 ppm oxygen (5,1 - 8,6)

tank temp = 19,13°C (18,0 - 19,9)

ANALYSIS: The first 41 days of operation were steady at the average COD of 379 mg/ℓ and X equal to 1020 mg/ℓ. The cell concentration then decreased reapidly to 310 mg/ℓ, and the COD rose to values greater than 1200 mg/ℓ. The tank recovered on day 76.

TANK NO: 7 TANK VOLUME: 5,800 ℓ

SUBSTRATE: Manucol

TANK DESCRIPTION: Flow through system

HISTORY OF MICRO-ORGANISMS: Previously operated as Tank No 5.

PERIOD OF OPERATION: As for Tank No 4. The hydraulic age (equal to the sludge age) was 2,37 days.

EXPERIMENTAL DATA: Tabulated in Table 7.

TABLE B.7

EXPERIMENTAL DATA FOR TANK NO: 7 OPERATING ON MANUCOL AS SUBSTRATE

TIME (days)	COD-g.f. (mg/ℓ)	COD-milli (mg/ℓ)	X (mg/ℓ)	pH
O-start				
4	439		1083	
6	200		1113	
8	200	85	1218	7,40
11	201	156	1331	
13		135	1312	7,20
15	243	198	1476	
16	198	198	1376	
19	246	246	1395	6,92
22	336		1008	
24	372		915	
31	432		875	5,9

Additional Information:

As for Tank No 5.
The COD results, although different initially, were equal to the COD g.f. results towards the end of the run.

TANK NO: 8 TANK VOLUME: 5,605ℓ

SUBSTRATE: Manucol

TANK DESCRIPTION: Flow through system.

HISTORY OF MICRO-ORGANISMS: Previously operated as Tank No. 6

PERIOD OF OPERATION: 1.5.74 - 9.6.74

OPERATING CONDITIONS: As for Tank No. 4. The hydraulic age (equal to the sludge age) was 9 days.

EXPERIMENTAL DATA: Tabulated in Table 8.

TABLE B.8

EXPERIMENTAL DATA FOR TANK NO: 8 OPERATING ON MANUCOL AS SUBSTRATE

TIME (days)	COD-g.f. (mg/ℓ)	COD-milli (mg/ℓ)	X (mg/ℓ)	pH
O-start				
4	214		1518	
6	200		1495	
8	215	125	1375	8,50
11	198	171	1483	
13	195	156	1382	8,90
15	240	240	1420	
16	246	213	1436	
19	255	231	1396	8,82
22	297		1465	
24	306		1362	
31	321	315	1468	9,00
37			1470	
39	306	285		

Additional Information for Tank 8:

As for Tank No 6.

$\frac{COD_{mi}}{COD_{gf}} = 0,866$

ANALYSIS: As the hydraulic and sludge ages were equal, the long sludge age meant that very small volumes of feed entered the reactor. Hence it would have taken a very long time for the cells to reach a steady state value.

TANK NO: 9 TANK VOLUME: 5,810ℓ

SUBSTRATE: Manucol

TANK DESCRIPTION: Flow through system

HISTORY OF MICRO-ORGANISMS: Previously operating as Tank No 4.

PERIOD OF OPERATION: 14.6.74 - 1.7.74

OPERATING CONDITIONS: The hydraulic residence time (equal to sludge age) was set by the automatic feeding system. Feed twice an hour,15 mins being allowed for the feeder to fill, and 30 mins to discharge. The hydraulic age was 1,01 days.
The feed concentration was 3000 mg/ℓ COD

EXPERIMENTAL DATA: Tabulated in Table 9.

TABLE B.9

EXPERIMENTAL DATA FOR TANK NO: 9 OPERATING ON MANUCOL AS SUBSTRATE

TIME (days)	COD-g.f. (mg/ℓ)	COD-milli (mg/ℓ)	X (mg/ℓ)	pH
O-start				
4	960		1290	8,18
6	.			8,10
8	340	325	1239	
10	400		1039	8,05
12	235	235	1001	
13	280	268	1101	
14	288	248	884	
15				7,75
17	232	200	864	

Additional Information for Tank 9:

S_o = 3000 mg/ℓ COD

$\frac{COD_{mi}}{COD_{gf}}$ = 0,927

ANALYSIS: The COD decreased slowly until it reached a steady value of 258 mg/ℓ. This COD was maintained between days 12 - 17. The steady state COD based on the millipore determinations was 238 mg/ℓ.
The cell concentration decreased from 1239 mg/ℓ on day 8 to 864 mg/ℓ on day 17. A steady state was maintained for days 14 - 17 at 874 mg/ℓ.

TANK NO: 10 TANK VOLUME: 5,810 ℓ

SUBSTRATE: Manucol

TANK DESCRIPTION: Flow through system.

HISTORY OF MICRO-ORGANISMS: Previously operating as Tank No 9

PERIOD OF OPERATION: 2.7.74 - 9.7.74

OPERATING CONDITIONS: The hydralic residence time (equal to sludge age) was set by the automatic feeding system. Feed four times an hour, 10 mins being allowed for the feeders to fill, and 5 mins to discharge. The hydraulic age was 0,50 days.
The feed concentration was 3000 mg/ℓ

EXPERIMENTAL DATA: Tabulated in Table 10

TABLE B.10

EXPERIMENTAL DATA FOR TANK NO: 10 OPERATING ON MANUCOL AS SUBSTRATE

TIME (days)	COD-g.f. (mg/ℓ)	COD-milli (mg/ℓ)	X (mg/ℓ)	pH
O-start				
1		800	892	7,28
2	250	320	1284	
3	324	260	1212	
6	548	536	886	
7	408	392	1021	

Additional Information for Tank 10:

S_o = 3000 mg/ℓ COD

ANALYSIS: The fluctuations in the results obtained at this setting were too considerable for any reliable steady state estimation to be made.

TANK NO: 11 TANK VOLUME: 5,800 ℓ

SUBSTRATE: Manucol

TANK DESCRIPTION: Flow through system.

HISTORY OF MICRO-ORGANISMS: Previously operated as Tank No 7

PERIOD OF OPERATION: 14.6.74 - 1.7.74

OPERATING CONDITIONS: As for Tank No. 9

The hydraulic age (equal to sludge age) was 1,73 days.

EXPERIMENTAL DATA: Tabulated in Table 11.

TANK NO: 12 TANK VOLUME: 5,800 ℓ

SUBSTRATE: Manucol

TANK DESCRIPTION: Flow through system.

HISTORY OF MICRO-ORGANISMS: Previously operated as Tank No 11.

OPERATING CONDITIONS: As for Tank No 10. The hydraulic age (equal to sludge age) was 0,86 days.

EXPERIMENTAL DATA: Tabulated in Table 12.

TABLE B.11

EXPERIMENTAL DATA FOR TANK NO: 11 OPERATING ON MANUCOL AS SUBSTRATE

TIME (days)	COD-g.f. (mg/ℓ)	COD-milli (mg/ℓ)	X (mg/ℓ)	pH
O-start				
4	375		693	7,30
6				7,20
8	400	325	712	
10	315		813	7,38
12	210	170	975	
13	208	200	941	
14	220	220	879	
15				6,90
17	408	128	630	

Additional Information for Tank 11:

S_o = 3000 mg/ℓ COD

$\frac{COD_{mi}}{COD_{gf}}$ = 0,896

ANALYSIS: The COD fluctuated about the average value of 305 mg/ℓ and the average cell concentration was estimated to be 800 mg/ℓ. The average millipore COD was 273 mg/ℓ.

TABLE B.12

EXPERIMENTAL DATA FOR TANK NO: 12 OPERATING ON MANUCOL AS SUBSTRATE

TIME	COD-g.f. (mg/ℓ)	COD-milli (mg/ℓ)	X (mg/ℓ)	pH
O-start				
1	1480		317	5,80
2	1380	1380	298	
3	620		841	
6	570		841	
7	280	210	736	

Additional Information for Tank 12:

S_o = 3000 mg/ℓ COD

ANALYSIS: The COD values were quite scattered, but the cell concentration appeared to have the same average value as the previous steady state value i.e. 800 mg/ℓ.

TANK NO: 13 TANK VOLUME: 5,605 ℓ

SUBSTRATE: Manucol

TANK DESCRIPTION: Flow through system.

HISTORY OF MICRO-ORGANISMS: Previously operated as Tank No 8.

PERIOD OF OPERATION: 14.6.74 - 1.7.74

OPERATING CONDITIONS: As for Tank No 9. The hydraulic age (equal to sludge age) was 2,92 days.

EXPERIMENTAL DATA: Tabulated in Table 13

TANK NO: 14 TANK VOLUME: 5,605 ℓ

SUBSTRATE: Manucol

TANK DESCRIPTION: Flow through system

HISTORY OF MICRO-ORGANISMS: Previously operated as Tank No 13.

PERIOD OF OPERATION: 2.7.74 - 9.7.74

OPERATING CONDITIONS: As for Tank No 10. The hydraulic age (equivalent to sludge age) was 1,46 days.

EXPERIMENTAL DATA: Tabulated in Table 14.

TABLE B.13

EXPERIMENTAL DATA FOR TANK NO: 13 OPERATING ON MANUCOL AS SUBSTRATE

TIME (days)	COD-g.f. (mg/ℓ)	COD-milli (mg/ℓ)	X (mg/ℓ)	pH
O-start				
4	285		1293	7,80
6				6,90
8	360	315	972	
10	400		790	6,89
12	305	235	811	
13	308	308	784	
14	320	288	761	
15				7,01
17	300	276	703	

Additional Information for Tank 13:

$\frac{COD_{mi}}{COD_{gf}} = 0,893$

ANALYSIS: This steady state is better than for some of the others because the population appeared to be at all times more homogeneous i.e. at no time were there any flocs which settled out in the tanks. The steady state values were COD = 310 mg/ℓ, COD (millipore) = 297 mg/ℓ, X = 790 mg/ℓ.

TABLE B.14

EXPERIMENTAL DATA FOR TANK NO: 14 OPERATING ON MANUCOL AS SUBSTRATE

TIME (days)	COD-g.f. (mg/ℓ)	COD-milli (mg/ℓ)	X (mg/ℓ)	pH
O-start				
1	500	250	642	7,55
2	310	320	805	
3	296	324	819	
6	424	164	791	
7	264	168	775	

Additional Information for Tank 14:

S_o = 3000 mg/ℓ COD

$\frac{COD_{mi}}{COD_{gf}} = 0,672$

ANALYSIS: An excellent steady state was achieved for the same reasons as discussed for Tank 13. Steady state was: COD = 300mg/ℓ, COD (millipore) = 227 mg/ℓ and X = 790 mg/ℓ.

TANK NO: 15 TANK VOLUME: 5,810 ℓ

SUBSTRATE: Manucol

TANK DESCRIPTION: Flow through system

HISTORY OF MICRO-ORGANISMS: Previously operated as Tank No 10.

PERIOD OF OPERATION: 17.7.74 - 9.9.74

OPERATING CONDITIONS: The hydraulic residence time (equal to sludge age) was set by the automatic feeding system. Feed once an hour, 30 mins being allowed for the feeders to fill, and 30 mins to discharge. The hydraulic age was 2,02 days.

EXPERIMENTAL DATA: Tabulated in Table 15.

TANK NO: 16 TANK VOLUME: 5,800 ℓ

SUBSTRATE: Manucol

TANK DESCRIPTION: Flow through system.

HISTORY OF MICRO-ORGANISMS: Previously operated as Tank No 12.

PERIOD OF OPERATION: 17.7.74 - 9.9.74

OPERATING CONDITIONS: As for Tank No 16. The hydraulic age (equal to sludge age) was 3,45 days.

EXPERIMENTAL DATA: Tabulated in Table 16.

TABLE B.15

EXPERIMENTAL DATA FOR TANK NO: 15 OPERATING ON MANUCOL AS SUBSTRATE

TIME (days)	COD-g.f. (mg/ℓ)	COD-millι (mg/ℓ)	X (mg/ℓ)	pH
0-start				
9	96	80	374	
11	120	120	343	
14	140		313	
17	141		258	6,95
21	123	93	231	
24	135	120	250	6,71
32	102	90	270	
35	114	96	282	
49	153	123	282	

Additional Information for Tank No 15:

S_o = 910 mg/ℓ COD

$\frac{COD_{mi}}{COD_{gf}}$ = 0,877

ANALYSIS: Steady operation was obtained for days 14 - 49. The steady state estimates were COD = 130 mg/ℓ, COD (millipore) = 103 mg/ℓ and X = 262 mg/ℓ.

TABLE B.16

EXPERIMENTAL DATA FOR TANK NO: 16 OPERATING ON MANUCOL AS SUBSTRATE

TIME (days)	COD-g.f. (mg/ℓ)	COD-millι (mg/ℓ)	X (mg/ℓ)	pH
9	156	136	446	
11	150	150	320	
14	159		236	
17	156		194	6,40
21	396	381	98	
24	364	370	236	6,80
32	135	144	228	
35	144	127	203	
49	153	132	217	

Additional Information for Tank 16:

S_o = 910 mg/ℓ COD

$\frac{COD_{mi}}{COD_{gf}}$ = 0,980

ANALYSIS: The results recorded for day 21 are discarded; a reliable estimate of the steady state values are COD = 150 mg/ℓ, COD (millipore) = 138 mg/ℓ, and X = 219 mg/ℓ

TANK NO: 17 TANK VOLUME: 5,605 ℓ

SUBSTRATE: Manucol

TANK DESCRIPTION: Flow through system

HISTORY OF MICRO-ORGANISMS: Previously operated as Tank No 14

PERIOD OF OPERATION: 17.7.74 - 9.9.74

OPERATING CONDITIONS: As for Tank no 16. The hydraulic age (equal to sludge age) was 5.84 days.

EXPERIMENTAL DATA: Tabulated in Table 17.

TABLE B.17

EXPERIMENTAL DATA FOR TANK NO: 17 OPERATING ON MANUCOL AS SUBSTRATE

TIME (days)	COD-g.f. (mg/ℓ)	COD-millí (mg/ℓ)	X (mg/ℓ)	pH
O-start				
4	370			
9	204	168	293	
11	342	321	194	
14	548		119	
17	330		281	6,80
21	130	135	190	
24	234	147	181	6,12
32	156	150	223	
35	144	133	220	
38	150	132	197	
49	204	183	316	

Additional Information for Tank 17:

S_o = 910 mg/ℓ COD

$\frac{COD_{mi}}{COD_{gf}}$ = 0,886

ANALYSIS: Steady operation was obtained between days 17 - 49 at COD = 170 mg/ℓ, COD (millipore) = 147 mg/ℓ, and X = 201 mg/ℓ.

TANK NO: 26 TANK VOLUME: 5,800 ℓ

SUBSTRATE: Mixed

TANK DESCRIPTION: Flow through system

HISTORY OF MICRO-ORGANISMS: Previously operated as Tank no 23

PERIOD OF OPERATION: 12.2.74 - 9.6.74

OPERATING CONDITIONS: As for Tank no 4. The hydraulic residence time was 4,74 days. Readjusted on day 80 to 4,65. The feed concentration was 3095 mg/ℓ COD.

EXPERIMENTAL DATA: Tabulated in Table 26.

TABLE B.26

EXPERIMENTAL DATA FOR TANK NO: 26

OPERATING ON A MIXED SUBSTRATE

TIME (days)	COD-g.f. (mg/ℓ)	COD-milli (mg/ℓ)	X (mg/ℓ)	pH
0-start				
2	461		1067	6,50
3				6,68
4	110		1155	
6	146		1414	6,86
7	106		1366	
8	116		1400	6,82
9	102		1515	6,72
10	104	104	1621	
13			1883	
14	134			
22				5,72
23	779		986	
24			1220	5,84
27	276		1174	5,70
28	264		1061	
41			1264	
47	374		1152	4,86
49				5,19
51	200		1255	5,28
52				5,28
55	300		916	6,32
76	372		944	6,80
82	212		1017	
84	165		996	
86	160	100	808	5,70
89	117	135	828	
91				5,82
93	180	180	675	
94	162	150	736	
97	165	132	907	
100	267		932	
102	270		1003	
109	276	246	1128	6,20
112	183	171	1132	
115	207		824	
117	156	150		

Additional Information for Tank 26:

S_o	= 3095 mg/ℓ COD (3000 - 3300)
% volatility	= 87,26% (85,60 - 90,60)
$\frac{COD_{mi}}{COD_{gf}}$	= 0,921
[O]	= 5,8 ppm oxygen (2,6 - 8,0)
tank temp	= 18,85°C (18,3 - 19,2)

ANALYSIS: The effects of bad mixing are evident for the first 10 days of operation, when X built up from 1100 mg/ℓ to 1600 mg/ℓ. A feature of this period is that, although this buildup of solids indicated a very long solids residence time, the COD remained almost constant at about 100 mg/ℓ.

After the first 10 days, the variation of the COD and X results was within a narrow range, and reasonable extimates of steady state values could be made as follows:

R_β (d)	COD (mg/ℓ)	COD (millipore) (mg/ℓ)	X (mg/ℓ)
4,74	270	249	1076
4,65	218	201	988

TANK NO: 27 TANK VOLUME: 5,780ℓ

SUBSTRATE: Mixed

TANK DESCRIPTION: Flow through system

HISTORY OF MICRO-ORGANISMS: Previously operated as Tank No 24.

PERIOD OF OPERATION: 12.2.74 - 9.6.74

OPERATION CONDITIONS: As for tank no. 4. The hydraulic residence time was 4,69 days. Adjusted on day 80 to 4.62 days. The feed conc was 3095 mg/ℓ COD

EXPERIMENTAL DATA: Tabulated in Table 27.

TABLE B.27

EXPERIMENTAL DATA FOR TANK NO: 27

OPERATING ON A MIXED SUBSTRATE

TIME (days)	COD-g.f. (mg/ℓ)	COD-milli (mg/ℓ)	X (mg/ℓ)	pH
0 - start				
2	264		1338	5,50
3				5,35
4			1123	
6	162		1397	5,72
7	168		1381	
8	192		1353	5,28
9	187		1395	5,38
10	179	172	1286	
13			1170	
14	384		1082	
22				6,42
23	258		1339	
24	210		1530	6,38
27	213		1616	6,00
28	204		1562	
47	338		572	7,16
49				7,19
51	196		869	7,11
52				7,14
55	453		769	6,69
76	308		960	6,90
82	244		966	
84	180		796	
86	160	95	614	5,50
89	124	117	633	
91				6,20
93	228	204	551	
94	153	138	619	
97	240	192	712	6,30
100	246		692	
102	246		737	
109	228	204	887	6,25
112	183		846	
115	126		609	
117	81	78	•	

Additional Information for Tank 27:

S_o = 3095 mg/ℓ COD (3000 - 3300)

% volatility = 88,55% (85,50 - 94,77)

$\frac{COD_{mi}}{COD_{gf}}$ = 0,869

[O] = 5,4 ppm oxygen (4,6 - 7,2)

tank temp = 18,77°C (18,2 - 19,2)

ANALYSIS: The high cell concentrations at the beginning were due to inadequate mixing. The results for days 51 - 112 can be averaged to give the results: R_s=4,65d, COD=228 mg/ℓ, COD (millipore) = 198 mg/ℓ and X=761 mg/ℓ.

TANK NO: 28 TANK VOLUME: 5,905ℓ

SUBSTRATE: Mixed

TANK DESCRIPTION: Flow through system

HISTORY OF MICROORGANISMS: Previously operated as Tank No 25

PERIOD OF OPERATION: 12.2.74 - 9.6.74

OPERATING CONDITIONS: As for Tank no. 4. The hydraulic residence time was 4,82 days. Adjusted on day 80 to 4,60 days. The feed conc was 3095 mg/ℓ COD.

EXPERIMENTAL DATA: Tabulated in Table 28.

TABLE B.28

EXPERIMENTAL DATA FOR TANK NO: 28

OPERATING ON A MIXED SUBSTRATE

TIME (days)	COD-g.f. (mg/ℓ)	COD-milli (mg/ℓ)	X (mg/ℓ)	pH
0 - start				
2	543		1020	6,05
3				6,11
4	127		1083	
6	143		1097	6,08
7	146		1053	
8	148		1082	6,00
9	148		1128	6,02
10		139	1079	
13			1242	
14	171		1326	
23	182		1423	5,92
24	163		1495	5,90
27	168		1658	5,45
28	170		1697	
47	331		647	7,18
49				7,19
51	168		769	7,18
52				7,20
55	410		885	7,18
76	261		935	6,65
82	157		894	
84	125		760	
86	125	80	934	6,32
89	129	135	949	
91	123	123	851	6,10
93	183	177	862	
94	171	144	617	
97	180	195	1016	6,28
100	255		878	
102	291		864	
109	246	246	1013	6,38
112	168	162	1066	
115	90		764	
117	144	138		

Additional Information for Tank 28:

S_o = 3095 mg/ℓ COD (3000 - 3300)

% volatility = 87,14% (79,41 - 92,50)

$\frac{COD_{mi}}{COD_{gf}}$ = 0,973

[O] = 6,3 ppm oxygen (4,4 - 7,2)

tank temp = 18,82°C (18,30 - 19,0)

ANALYSIS: The mixing was improved after the first 3 weeks of operation and reasonable estimates could be made of the steadystate: R_s = 4,60d, COD = 187 mg/ℓ, COD (millipore) = 187 mg/ℓ, X = 881 mg/ℓ.

TANK NO: 29 TANK VOLUME: 5,800ℓ

SUBSTRATE: Mixed

TANK DESCRIPTION: Flow through system

HISTORY OF MICROORGANISMS: Previously operated as tank No 26.

PERIOD OF OPERATION: 14.6.74 - 1.7.74

OPERATING CONDITIONS: As for Tank No. 9. The hydraulic residence time was 1,025 days. The feed concentration was 3000 mg/ℓ COD.

EXPERIMENTAL DATA: Tabulated in Table 29.

TABLE B.29

EXPERIMENTAL DATA FOR TANK NO: 29

OPERATING ON A MIXED SUBSTRATE

TIME (days)	COD-g.f. (mg/ℓ)	COD-milli (mg/ℓ)	X (mg/ℓ)	pH
0-start				
4	1755		2726	3,40
6				3,75
8	360		1305	
10	150		1317	6,67
12	200	135	1180	
13	160	148	1392	
14	148	148	1292	
15				6,08
17	552	556	1099	

Additional information for Tank 29:

$\frac{COD_{mi}}{COD_{gf}}$ = 0,902

ANALYSIS: Steady state operation was attained at a COD of 160 mg/ℓ, COD (millipore) = 160 mg/ℓ and X = 1305 mg/ℓ.

TANK NO: 31 TANK VOLUME: 5,780ℓ

SUBSTRATE: Mixed

TANK DESCRIPTION: Flow through system.

HISTORY OF MICROORGANISMS: Previously operated as tank No 27.

PERIOD OF OPERATION: 14.6.74 - 1.7.74

OPERATING CONDITIONS: As for Tank No 9. The hydraulic residence time was 2,51 days. The feed concentration was 3000 mg/ℓ COD.

EXPERIMENTAL DATA: Tabulated in Table 31.

TABLE B.31

EXPERIMENTAL DATA FOR TANK NO: 31

OPERATING ON A MIXED SUBSTRATE

TIME (days)	COD-g.f. (mg/ℓ)	COD-millí (mg/ℓ)	X (mg/ℓ)	pH
0-start				
4	1300		768	4,0
6				5,1
8	225	185	1472	
10	480		1212	7,11
12	100	125	1486	
13	128	108	1377	
14	168	140	1284	
15				6,58
17	116	100	1317	

Additional information for Tank no 31:

$$\frac{COD_{mi}}{COD_{gf}} = 0,922$$

ANALYSIS: The COD reached a steady value of 120 mg/ℓ, which was equal to the millipore COD. The cell concentration oscillated somewhat and reached a steady state at 1320 mg/ℓ.

TANK NO: 30 TANK VOLUME: 5,800ℓ

SUBSTRATE: Mixed

TANK DESCRIPTION: Flow through system.

HISTORY OF MICROORGANISMS: Previously operated as tank No. 29.

PERIOD OF OPERATION: 2.7.74 - 9.7.74

OPERATING CONDITIONS: As for Tank no 10. The hydraulic residence time was 0,513 days. The feed conc was 3000 mg/ℓ COD.

EXPERIMENTAL DATA: Tabulated in Table 30.

TABLE B.30

EXPERIMENTAL DATA FOR TANK NO: 30

OPERATING ON A MIXED SUBSTRATE

TIME (days)	COD-g.f. (mg/ℓ)	COD-millí (mg/ℓ)	X (mg/ℓ)	pH
0-start				
1	1380	1330	823	4,50
2	1380	1390	490	
3	1360	1360	674	
6	1270	1210	878	

Additional information for Tank No 30:

$$\frac{COD_{mi}}{COD_{gf}} = 0,981$$

ANALYSIS: The cell concentration dropped to a very low value and then increased. The COD followed the inverse of this. This tank was not operated for a sufficient time for steady state conditions to be confidently predicted.

TANK NO: 32 TANK VOLUME: 5,750ℓ

SUBSTRATE: Mixed

TANK DESCRIPTION: Flow through system

HISTORY OF MICROORGANISMS: Previously operated as tank no 31.

PERIOD OF OPERATION: 2.7.74 - 9.7.74

OPERATING CONDITIONS: As for tank no 10. The hydraulic residence time was 1,255 days. The feed conc was 3000 mg/ℓ COD.

EXPERIMENTAL DATA: Tabulated in Table 32.

TABLE B.32

EXPERIMENTAL DATA FOR TANK NO: 32

OPERATING ON A MIXED SUBSTRATE

TIME (days)	COD-g.f. (mg/ℓ)	COD-milli (mg/ℓ)	X (mg/ℓ)	pH
O-start				
1	220	240	1283	6,50
2	190	190	1105	
3	204	148	1281	
6	168	148	1574	
7	148	128	1412	

Additional information for Tank no 32:

$$\frac{COD_{mi}}{COD_{gf}} = 0,912$$

ANALYSIS: The steady state values were estimated to be COD = 200 mg/ℓ, COD (millipore) = 200 mg/ℓ, and X = 1280 mg/ℓ.

TANK NO: 33 TANK VOLUME: 5,905 ℓ

SUBSTRATE: Mixed

TANK DESCRIPTION: Flow through system.

HISTORY OF MICROORGANISMS: Previously operated as tank no 28.

PERIOD OF OPERATION: 14.6.74 - 1.7.74

OPERATING CONDITIONS: As for Tank No. 9. The hydraulic residence time was 1,86 days. The feed conc was 3000 mg/ℓ COD.

EXPERIMENTAL DATA: Tabulated in Table 33.

TABLE B.33

EXPERIMENTAL DATA FOR TANK NO: 33

OPERATING ON A MIXED SUBSTRATE

TIME (days)	COD-g.f. (mg/ℓ)	COD-milli (mg/ℓ)	X (mg/ℓ)	pH
O-start				
4	1380		1522	3,9
6				5,9
8	300	185	1288	
10	200		1328	7,41
12	170	160	1279	
13	200	200	1307	
14	184	184	1252	
15				6,50
17	108	122	1314	

Additional information for Tank No. 33:

$$\frac{COD_{mi}}{COD_{gf}} = 1,0$$

ANALYSIS: An excellent steady state condition was achieved at a COD of 185 mg/ℓ, COD(millipore) = 185 mg/ℓ and X = 1300 mg/ℓ.

TANK NO: 34 TANK VOLUME: 5,905ℓ

SUBSTRATE: Mixed

TANK DESCRIPTION: Flow through system

HISTORY OF MICROORGANISMS: Previously operated as tank No. 33

PERIOD OF OPERATION: 2.7.74 - 9.7.74

OPERATING CONDITIONS: As for tank no 10. The hydraulic residence time was 0,831 days. The feed conc was 3000 mg/ℓ COD.

EXPERIMENTAL DATA: Tabulated in Table 34.

TABLE B.34

EXPERIMENTAL DATA FOR TANK NO: 34

OPERATING ON A MIXED SUBSTRATE

TIME (days)	COD-g.f. (mg/ℓ)	COD-milli (mg/ℓ)	X (mg/ℓ)	pH
O-start				
1	455	450	1213	6,80
2	210	190	1240	
3	224	140	1447	
6	220	148	1231	
7	220	168	1098	

Additional information for Tank No. 34:

$$\frac{COD_{mi}}{COD_{gf}} = 0,791$$

ANALYSIS: Reliable steady state operation was achieved at COD = 220 mg/ℓ, COD (millipore) = 162 mg/ℓ and X = 1235 mg/ℓ.

TANK NO: 35 TANK VOLUME: 5,800ℓ

SUBSTRATE: Mixed

TANK DESCRIPTION: Flow through system.

HISTORY OF MICROORGANISMS: Previously operated as tank no. 30.

PERIOD OF OPERATION: 17.7.74 - 9.9.74

OPERATING CONDITIONS: As for Tank No. 15. The hydraulic residence time was 2,05 days. The feed conc was 940 mg/ℓ COD.

EXPERIMENTAL DATA: Tabulated in Table 35.

TABLE B.35

EXPERIMENTAL DATA FOR TANK NO: 35

OPERATING ON A MIXED SUBSTRATE

TIME (days)	COD-g.f. (mg/ℓ)	COD-milli (mg/ℓ)	X (mg/ℓ)	pH
O-start				
4	60			
9	40	40	401	
11	57	57	349	
14	83		355	
17	98		365	6,40
21	98	96	683	
24	75	66	513	6,45
35	51	51	489	
38	45	45	474	
49	58	52	566	

Additional Information for Tank No. 35:

$$\frac{COD_{mi}}{COD_{gf}} = 0,974$$

ANALYSIS: For days 0 - 17, the average values were COD = 79 mg/ℓ, COD (millipore) = 77 mg/ℓ and X = 356 mg/ℓ. For days 24 - 49 the results were COD = 57 mg/ℓ and X = 511 mg/ℓ.

TANK NO: 36 TANK VOLUME: 5,780ℓ

SUBSTRATE: Mixed

TANK DESCRIPTION: Flow through system.

HISTORY OF MICROORGANISMS: Previously operated as tank No. 32.

PERIOD OF OPERATION: 17.7.74 - 9.9.74

OPERATING CONDITIONS: As for Tank No. 15. The hydraulic residence time was 3,65 days. The feed conc. was 940 mg/ℓ COD.

EXPERIMENTAL DATA: Tabulated in Table 36.

TABLE B.36

EXPERIMENTAL DATA FOR TANK NO: 36 OPERATING ON A MIXED SUBSTRATE.

TIME (days)	COD-g.f. (mg/ℓ)	COD-milli (mg/ℓ)	X (mg/ℓ)	pH
0 - start				
9	48	48	392	
11	57	60	338	
14	68		343	
17	78		464	6,25
21	87	90	575	
24	87	75	506	6,48
32	96	96	373	
35	60	67	386	
38	54	45	383	
49	52	52	364	

Additional Information for Tank No 36:

$$\frac{COD_{mi}}{COD_{gf}} = 1,0$$

ANALYSIS: The COD and X concentrations were steady for the entire period of operation, except between days 17 - 24, when the cell concentration increased above the average value. However, it soon returned to its former value, and a satisfactory steady state operation was achieved, with values COD = 62 mg/ℓ, COD (millipore) = 62 mg/ℓ and X = 368 mg/ℓ.

TANK NO: 37 TANK VOLUME: 5,905ℓ

SUBSTRATE: Mixed

TANK DESCRIPTION: Flow through system

HISTORY OF MICROORGANISMS: Previously operated as Tank No. 34.

PERIOD OF OPERATION: 17.7.74 - 9.9.74

OPERATING CONDITIONS: As for Tank No. 15. The hydraulic residence time was 5,13 days. The feed conc was 940 mg/ℓ COD.

EXPERIMENTAL DATA: Tabulated in Table 37.

TABLE B.37

EXPERIMENTAL DATA FOR TANK NO: 37 OPERATING ON A MIXED SUBSTRATE

TIME (days)	COD-g.f. (mg/ℓ)	COD-milli (mg/ℓ)	X (mg/ℓ)	pH
0-start				
4	100			
9	80	80	408	
11	78	60	355	
14	69		369	
17	80		473	6,30
21	78	78	591	
24		66	526	6,38
35	69	51	462	
38	75	45	407	
49	58	62	366	

Additional Information for Tank No 37:

$$\frac{COD_{mi}}{COD_{gf}} = 0,928$$

ANALYSIS: The COD was constant for the entire period of operation. The cell concentration was constant for about 15 days. It then increased and slowly decreased again to its former value, which it maintained for days 38 - 49. Hence a reliable estimate of the steady state values could be determined: COD = 68 mg/ℓ, COD(millipore) = 64 mg/ℓ and X = 381 mg/ℓ.

APPENDIX C

CELL CONCENTRATION CORRECTIONS

For some of the manucol experiments, solids concentration measurements were made gravimetrically using glass fibre and millipore filter papers, and also turbidometrically. A correlation was found to exist between millipore solids concentration and turbidity, as shown in Figure C.1. Effluent COD was measured on filtrates obtained from glass fibre and millipore filter papers, hence the contribution to the COD of those solids that passed through the glass fibre paper could be readily determined. In Figure C.2, ΔX is plotted against Δ COD, where

ΔX = millipore solids - glass fibre solids

Δ COD = glass fibre COD - millipore COD

A correlation exists, and the relationship is given by $\Delta X = 1{,}01\,\Delta \text{COD} + 9{,}1$. Thus 9 mg/$\ell$ of solids that pass through the glass fibre paper do not register as COD; then the relationship is 1 mg/ℓ of solids is equivalent to 1 mg/ℓ COD. The glass fibre gravimetric measurements of cell concentrations were corrected using this relationship.

The mixed substrates generally had a clean effluent as far as colloidal solids were concerned. This is evident from the data, which indicate that the glass fibre and millipore COD determinations were generally equal. Thus it was not necessary to correct for solids in the mixed substrates effluent.

FIGURE C.1 MANUCOL SOLIDS CORRELATION

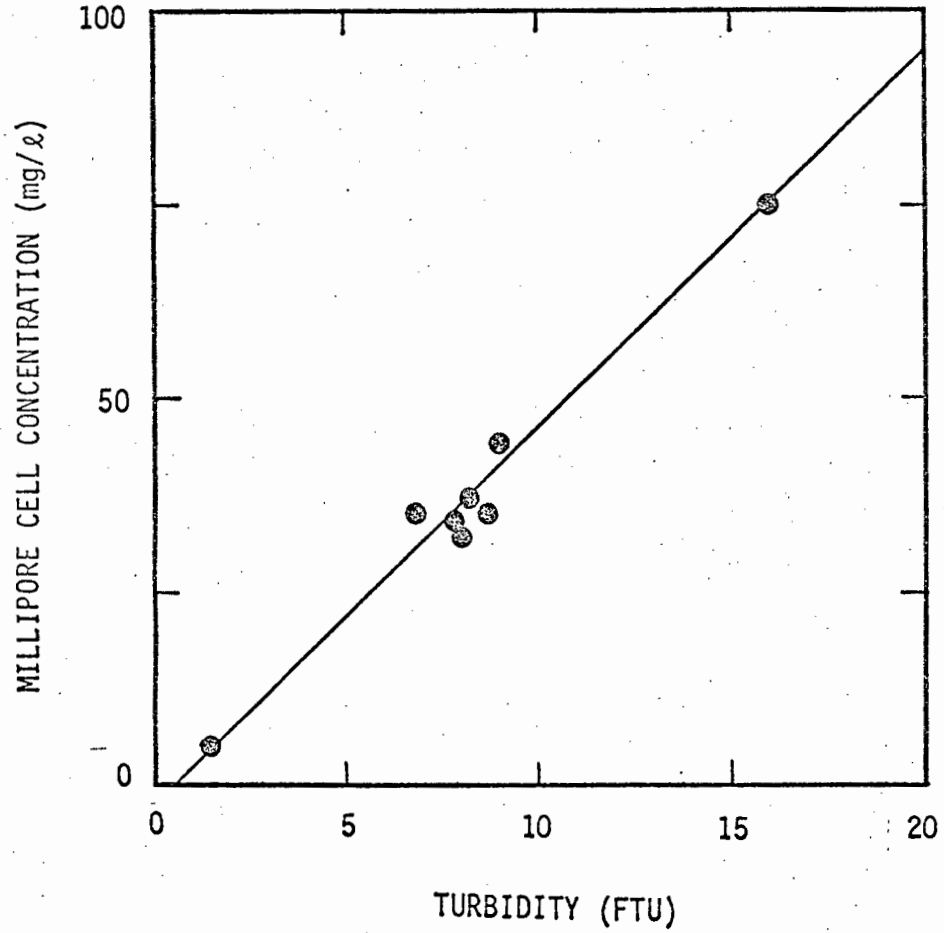
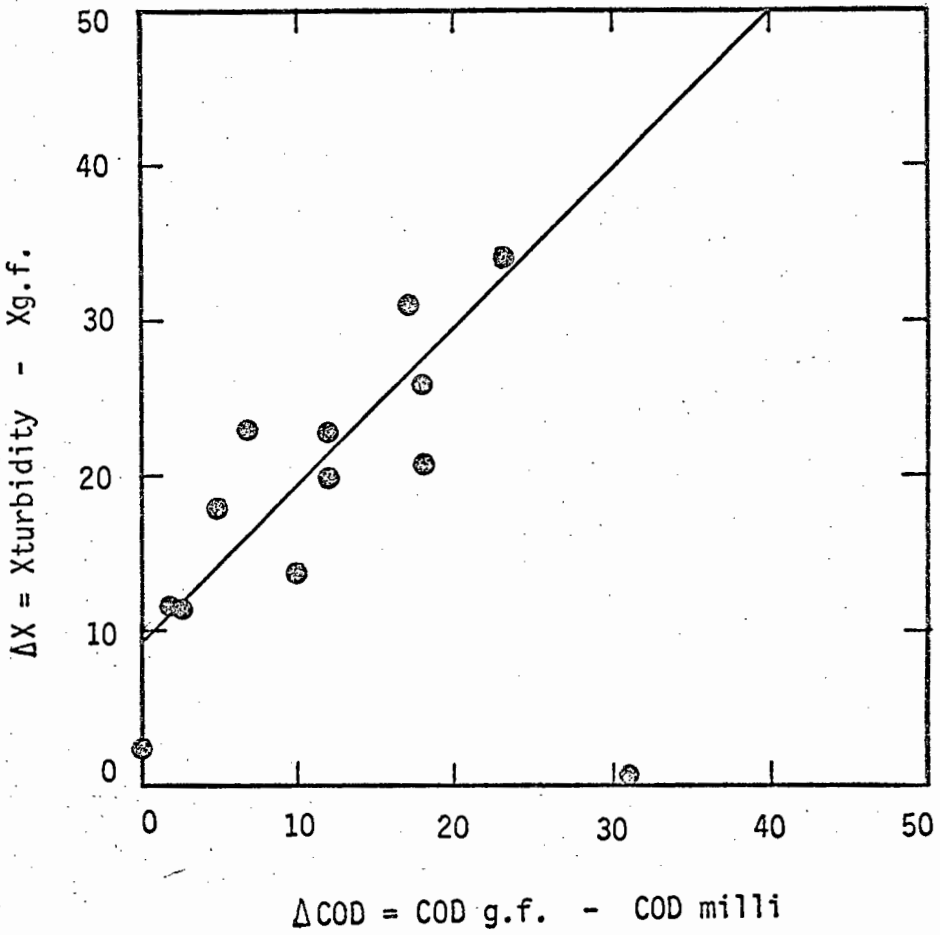


FIGURE C.2 CONTRIBUTION OF SOLIDS TO COD FOR MANUCOL



APPENDIX D

SIPHON DESIGN

Siphons were used on the flow-through tanks to prevent filtering of solids at the exit pipe. Several different designs of syphons were investigated. Some of these are shown in Figure D.1. A design such as is shown in Figure D.1(i) did not work as a meniscus of liquid formed in the siphon tube and was sucked over; hence there was never any siphoning of the mixed liquor. The design shown in Figure D.1(ii) was not efficient as solids tended to settle out in the bend. After much trial and error, the design shown in Figure D.1(iii) proved to be efficient and was adopted. The volume of liquid built up to the level d before any siphoning action took place. The tube e was open at both ends. Any air bubbles that entered the siphon were thus vented to the atmosphere, and did not transport liquid out of the tank through the siphon tube.

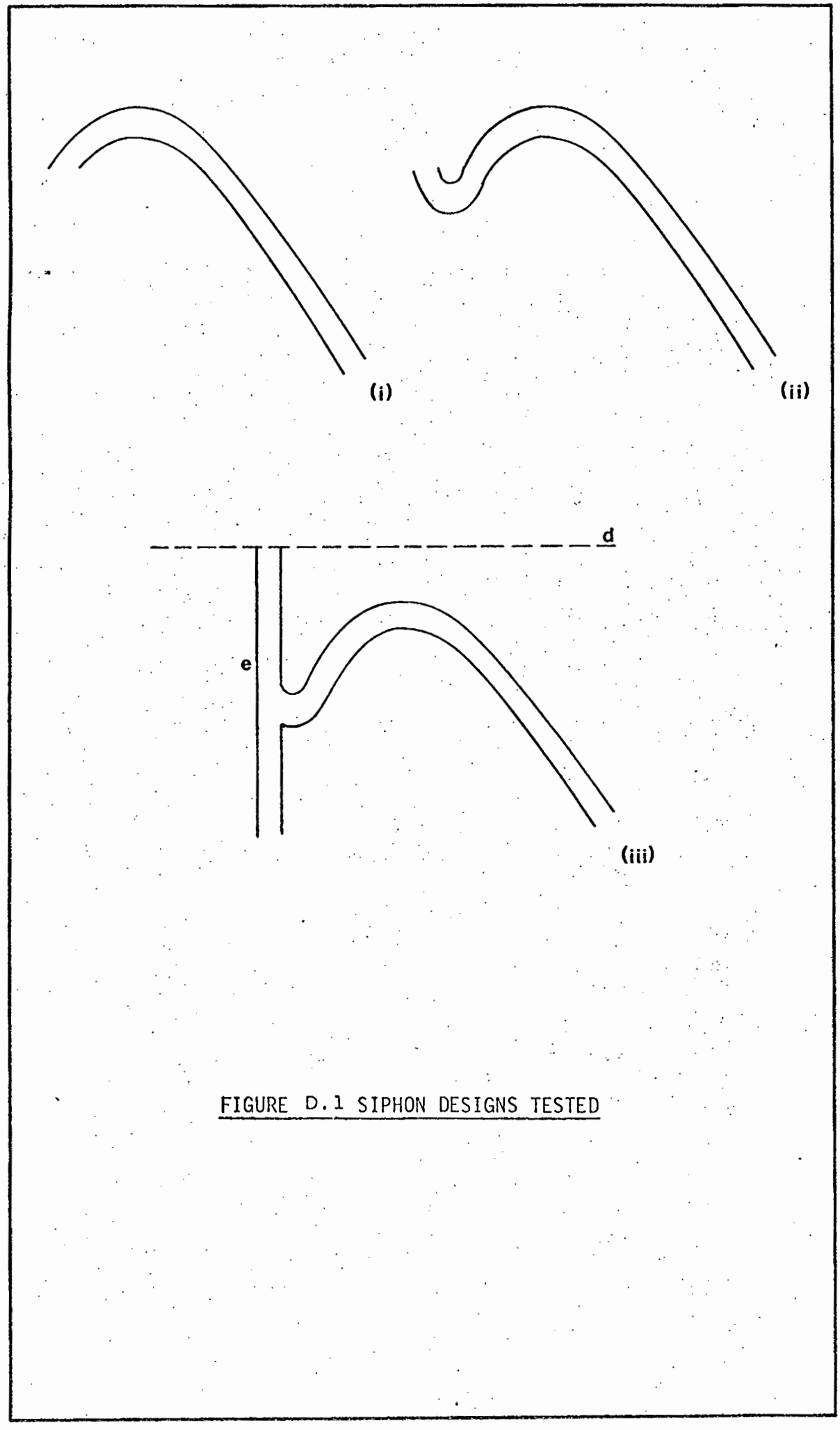


FIGURE D.1 SIPHON DESIGNS TESTED

TANK NO: 23 TANK VOLUME: 5,800 ℓ

SUBSTRATE: Mixed.

TANK DESCRIPTION: Settling compartment for effluent included.

HISTORY OF MICRO-ORGANISMS: Feed taken from batch acclimatised flask.

PERIOD OF OPERATION: 10.11.73 - 31.1.74

OPERATING CONDITIONS: As for Tank No. 1. The hydraulic residence time was 1.16 days. The feed concentration was 1040 mg/ℓ COD

EXPERIMENTAL DATA: Tabulated in Table No. 23

TABLE B.23

EXPERIMENTAL DATA FOR TANK NO: 23 OPERATING ON A MIXED SUBSTRATE

TIME (days)	COD (mg/ℓ)	X (mg/ℓ)	pH	SVI	X_e (mg/ℓ)	R_s (d)
0-start						
1	69	735	7,04			8-nominal
4			6,70			
6	174	1243				8
12	140	2059				8
13	156	2201	6,52			8
14	305	2284				8
16	500	1766	6,28	86	32	8
19	120	1725				8
25			5,60			
26	58	1966		96		7,18
27	72	2020	6,12	100		7,22
30		2083		86	72	7,27
31	115	1826	5,62	105		8,05
32			5,40		35	
33	224	1989	6,15	96	42	7,94
34	166	1977				16,32
36	91	1860		98		9,03
37		1959			16	9,05
38	155	1836	5,48	112	34	8,37
39	192	1843				8,55
40	229	1849			29	8,55
41	266	1701			125	6,00
42	309	1655				5,93
43	147	1627	5,02			5,89
45	169	1579				5,82
53					123	
54	162	1216				6,66
56	129	1495			66	7,07
57					37	
58	104	1678	5,98			9,34
59	91	1895				9,38
60					7	
61	146	1962			3	9,55
62	95	2084				9,44
63	73	2126	5,69	365	6	9,44
64	74	2109				9,44
65					6	
66	94	2082	5,49	446		8,82
67					24	
68					87	
69	102	1675	5,90	579		2,05
70	91	1203	6,25		747	1,87
71	71	1396			174	4,74
72	77	1462	6,29	650		6,13
73	73	1391				6,02
74					101	

Additional Information for Tank 23:

S_o = 1040 mg/ℓ COD (1000 - 1103)
% volatility = 89,96% (89,49 - 90,46)
DO = 5,42 ppm oxygen (4,0 - 6,95)
tank temp = 20,07°C (19,9 - 20,2)

ANALYSIS: Although the cell concentration fluctuated considerably, the COD remained practically constant throughout the entire period of operation. From about day 53 filamentous micro-organisms were detected in the tank. These became more predominant with time, and the tank contents changed from a green-brown colour to a progressively darker colour. The SVI values recorded for the initial period of operation were all about 100. Towards the end of this experiment the values increased rapidly, indicating the onset of filamentous growth.

It is noted that the filamentous growth had the effect of binding the micro-organisms together. This resulted in a reduction of the cell concentration in the clear effluent. The COD determination was then less subject to the effect of varying numbers of free swimming micro-organisms.

The steady state estimates were:

Period considered (days)	R_s (d)	COD (mg/ℓ)	X (mg/ℓ)	SVI
26 - 40	8,12	145	1930	97
58 - 66	9,34	97	2100	365

TANK NO: 24 TANK VOLUME: 5,780 ℓ

SUBSTRATE: Mixed

TANK DESCRIPTION: Settling compartment for effluent included.

HISTORY OF MICRO-ORGANISMS: Feed taken from batch acclimatised flask.

PERIOD OF OPERATION: 10.11.73 - 2.2.74

OPERATING CONDITIONS: As for Tank No. 1. The hydraulic residence time was 1.16 days. The feed concentration was 1040 mg/ℓ COD.

EXPERIMENTAL DATA: Tabulated in Table 24.

TABLE B.24

EXPERIMENTAL DATA FOR TANK NO: 24 OPERATING ON A MIXED SUBSTRATE

TIME (days)	COD (mg/ℓ)	X (mg/ℓ)	pH	SVI	X_e (mg/ℓ)	R_s (d)
0-start						
1	286	486	7,20			8-nominal
4			6,94			
6	149	944				8
12	160	1642				8
13	183	1688	5,98			8
14	191	1771				8
16	240	1714	5,84	70	51	8
19	89	1881				8
25			5,30			
26	66	2116		74		8,22
27	84	2002	5,60	89		8,17
30		2077		62	64	8,21
31	97	1839	5,70	72		8,09
32			5,55		33	
33	199	2079	6,18	63	50	7,41
34	111	2132				13,82
36	71	2042		68		8,17
37		2100			44	8,20
38	110	1836	5,69	60	35	8,31
39	140	1896				8,54
40	192	1906			29	8,55
41	318	1769			262	4,31
42	412	1499				3,86
43	386	1093	6.22			3,16
45	158	840				2,63
53					169	
54	168	629				2,46
56	162	703			220	2,67
57					206	
58	219	663	6,59			3,00
59	146	726				3,19
60					176	
61	144	865			156	3,85
62	97					
63	114	946	6,49		142	4,28
64	111	939				4,67
65					120	
66	111	992	6,37	28		6,10
67					69	
68					63	
69	91	1296	6,50	54		8,44
70	89	1373	6,50		22	8,50
71	99	1468			34	8,07
72	98	1354	6,60	124		7,73
73	103	1508				7,89
74					40	
75	90	1542	6,60			9,38
76	92	1668			5	9,40
77	130	1525				8,48
78	80	1506			25	8,46
79	84	1585		424		8,51
80			6,45			
81	110	1397			28	8,25
82	91	1518		494		8,35
83	35	1680	6,51			8,46
84	35	1304				8,17

Additional Information for Tank 24:

S_o = 1040 mg/ℓ COD (1000 - 1105)

% volatility = 86,22% (85,82 - 87,05)

DO = 4,77 ppm oxygen (2,62 - 6,95)

tank temp = 19,48°C (19,98 - 20,2)

ANALYSIS: As for Tank 23. Steady state estimates were:

Period considered (days)	R_s (d)	COD (mg/ℓ)	X (mg/ℓ)	SVI
25 - 40	8,19	90	2048	70
70 - 82	8,46	97	1550	274

TANK NO: 25 TANK VOLUME: 5,905 ℓ

SUBSTRATE: Mixed

TANK DESCRIPTION: Settling compartment for effluent included.

HISTORY OF MICRO-ORGANISMS: Seed taken from batch acclimatised flask.

PERIOD OF OPERATION: 10.11.73 - 31.1.74

OPERATING CONDITIONS: As for Tank No. 1. The hydraulic residence time was 1,18 days. The feed concentration was 1040 mg/ℓ COD.

EXPERIMENTAL DATA: Tabulated in Table 25.

TABLE B.25

EXPERIMENTAL DATA FOR TANK NO: 25 OPERATING ON A MIXED SUBSTRATE

TIME (days)	COD (mg/ℓ)	X (mg/ℓ)	pH	SVI	X_e (mg/ℓ)	R_s (d)
0-start						
1	144	404	7,12			8-nominal
4			6,98			
6	145	670				8
12	365	1177				8
13	373	1222	6,29			8
14	382	1470				8
16	515	1573	6,30	69	27	8
19	95	1256				8
25			5,50			
26	87	2732		72		7,51
27	89	2793	5,76	76		7,55
30		2551		80	88	7,40
31	103	2153	6,18	88		7,37
32			5,48		76	
33	203	2296	6,20	88	85	7,29
34	111	2283				17,50
36	78	2080		94		9,01
37		2362			23	9,10
38	179	2131	5,50	92	30	8,81
39	96	2087				8,98
40	149	2048			24	8,97
41	211	1894			60	7,79
42	143	1957				7,84
43	102	1985	6,20			7,86
53					242	
54	131	455				1,86
56	184	530			235	2,10
57					165	
58	134	653	6,89			7,92
59	109	883				8,35
60					19	
61	100	1138			73	6,41
62		1181				9.25
63	84	1287	6,70	74	9	9,30
64	88	1319				6,79
65					71	
66	83	1364	6,40	50		7,47
67					52	
68					159	
69	79	1078	6,19	46		5,91
70	83	1058	6,25		86	5,87
71	67	1053			32	7,85
72	80	1360	6,21	223		7,57
73	92	1594				7,83
74					49	
75	89	1359	6,11			7,57
76	92	1496				7,73

Additional Information for Tank 25:

S_o = 1040 mg/ℓ COD (1000 - 1105)
% volatility = 86,51% (83,80 - 88,67)
DO = 4,99 ppm oxygen (2,0 - 6,95)
tank temp = 19,94°C (19,8 - 20,2)

ANALYSIS: As for Tank 23. A feature of this tank is the COD data. Despite the large fluctuations in the cell concentration (of the order of six times), the COD remained constant throughout the entire period of operation.

For the period 60 - 76, the average values of the parameters were R_s = 7,71 d, COD = 84 mg/ℓ, X = 1600 mg/ℓ and SVI = 223. However, steady state operation was never achieved with this tank.